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Heffer/DC/USEPA/US@EPA, HPV-OC@listserv.epa.gov, HPVTechOther@listserv.epa.gov
Subject: HPV Test Plan and Robust Summaries Submission for Waxes and Related Materials Category - HPV
Registration Number

The American Petroleum Institute (API) is pleased to submit the subject documents on behalf of the Petroleum HPV Testing Group. The submission consists of a cover letter to Christine Whitman, the Waxes and Related Materials Category Test Plan, and separate files containing the corresponding Robust Summary in .pdf format and as an IUCLID export file.

Please acknowledge receipt of this note, attached pdf files 3), and IUCLID export file. Also, please contact me with any questions or comments regarding this submission.

Thank you,

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- Submission Letter.PDF



- Waxes Test Plan.PDF



- Waxes.exp



- Waxes Robust Summary.PDF

Petroleum HPV

August 6, 2002

Christine T. Whitman, Administrator
1101A
USEPA Headquarters
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460
Attention: Chemical Right-to-Know

**RE: HPV TEST PLAN AND ROBUST SUMMARIES SUBMISSION FOR THE WAXES AND
RELATED MATERIALS CATEGORY**

HPV CONSORTIUM #

Dear Ms. Whitman:

The American Petroleum Institute, on behalf of the Petroleum HPV Testing Group, is pleased to submit the Waxes and Related Materials Test Plan and Robust Summaries. Our consortium has chosen not to use the HPV Tracker system for submission of our test plans due to the complexity of petroleum substances categories and subsequent test plans. We are therefore submitting the test plan, as well as robust summaries directly to EPA to make available for public comment.

Electronic copies of the test plan (in .PDF format) and robust summaries (in .PDF format and as an IUCID export file) are accompanying this letter via email to the EPA HPV robust summary email address (<http://www.epa.gov/chemrlk/robsum.htm>). This submission is also being sent, via email, to the individuals listed below, including Mr. Charles Auer.

Please feel free to contact me (202-682-8344; twerdok@api.org) or Tom Gray (202-682-8480; grayt@api.org) with any comments or questions you may have regarding this submission.

Sincerely,

Lorraine Twerdok, Ph.D., DABT
Administrator, Petroleum HPV Testing Program

Cc: C. Auer, USEPA
R. Hefter, USEPA
O. Hernandez, USEPA
Petroleum HPV Testing Group Oversight Committee
Petroleum HPV Testing Group Technical Committee

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HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

WAXES AND RELATED MATERIALS CATEGORY

Submitted to the US EPA

by

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration #

August 6, 2002

TEST PLAN

WAXES AND RELATED MATERIALS CATEGORY

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PLAIN LANGUAGE SUMMARY

This test plan addresses the refinery streams and finished products involved in the production of petroleum waxes and related materials. The materials in this category are complex petroleum mixtures composed of hydrocarbons with carbon numbers ranging from C12 to C85, with the majority exceeding C20. All the materials in the category are solid or semi-solid at room temperature. The physical state (semi-solid to solid) and the number of carbon atoms in the petroleum waxes severely limit their bioavailability and their environmental distribution, breakdown, or conversion.

Based on the degree of processing and level of residual oil and impurities, the streams and products within this category can be divided into three subcategories:

1. Unfinished Wax (Slack Wax),
2. Refined/Finished Wax (Paraffin and/or Microcrystalline Wax), and
3. Petrolatum (Petroleum Jelly).

In addition to being the precursors to refined/finished waxes and petrolatum, slack waxes are generally limited to industrial applications such as lubricants and specialty cleaning/preservative products. Refined/finished waxes and petrolatum (petroleum jelly) have a large and varied number of industrial and consumer product applications. They may be used in lubricants, wire cables, candles, Vaseline, cosmetic, and food/drug applications.

Of the three subcategories of waxes, slack wax contains the greatest amount of base oil and impurities as well as having the largest variation of hydrocarbon molecules. Refined/finished waxes and petrolatum are produced from slack waxes by removing these impurities and base oils. Based on the existing data and the physical/chemical nature of these materials, the Testing Group expects the potential toxicity of any of the wax category members will arise primarily from the base oil component of the wax. The base oils will be addressed as a category in the Lubricating Oil Basestocks HPV Test Plan.

After evaluating the extensive toxicology database for the three sub-categories of waxes and using read-across information within the waxes sub-categories and the Lubricating Oil Basestocks HPV Test Plan, the Testing Group is proposing to perform selected toxicity testing on the least refined stream (slack wax) in order to fill any existing data gaps for the Waxes category. The Testing Group thinks the toxicity testing of slack wax will address the hazards of all **three** subcategories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components and highest concentration of **bioavailable/biologically** active components of all the materials addressed in this Test Plan. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken. The studies on slack wax will include:

1. Mammalian Toxicity - Repeated Dose Reproductive/Developmental Screening study (OECD 422)
2. Genetic Toxicity - *In Vitro* Bacterial Reverse Mutation (OECD 471) and *In Vivo* Mammalian Erythrocyte Micronucleus test (OECD 474)

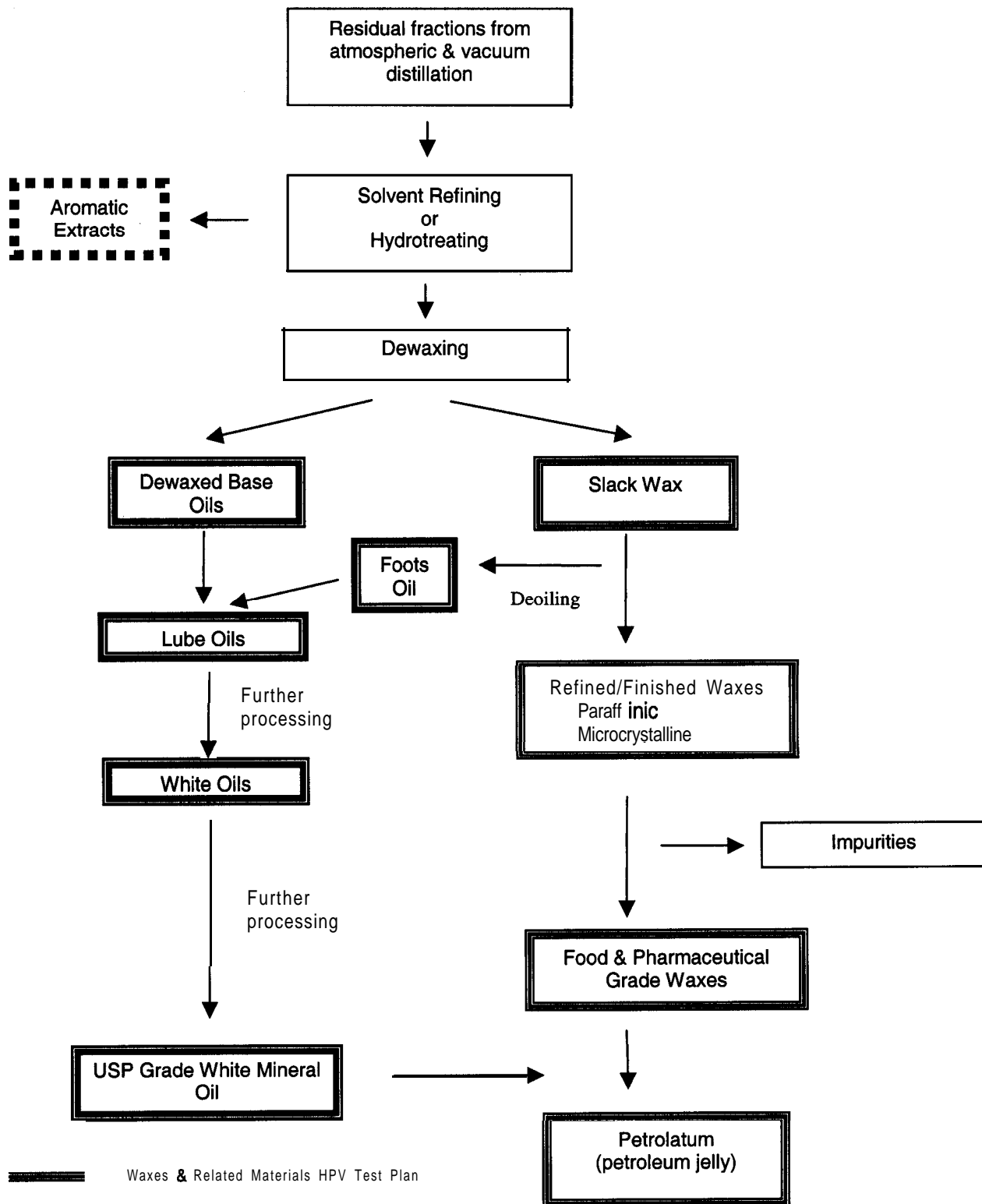
For all three sub-categories of waxes (slack waxes, finished/refined waxes and petrolatum), when physicochemical data did not exist or was impractical to obtain, calculated physicochemical and environmental data for selected constituents of waxes has been developed using the EPIWIN® computer model.

The Testing Group thinks the existing ecotoxicity data on waxes adequately describes their potential toxicity. Therefore the Testing Group is proposing no additional ecotoxicity testing on any of the waxes or related category members.

DESCRIPTION OF THE WAXES AND RELATED MATERIALS CATEGORY

The Waxes and Related Materials category includes both refinery streams and finished products. The materials in this category are complex petroleum mixtures composed of predominantly saturated hydrocarbons with carbon numbers ranging from C12 to C85, with the majority exceeding **C20**. Because they are complex mixtures, the petroleum waxes and related materials are typically defined by process history and product use specifications, not by detailed compositional information that identifies every specific individual molecular compound. All the materials in the category are solid or semi-solid at room temperature with very low volatility and water solubility values. As shown in the Figure 1, the materials included in the category are produced by a series of processing steps that separate the wax and oil portions of selected refinery streams. If present, the biologically available/active impurities such as **PAC/PNA** (polycyclic aromatic **compounds/polynuclear** aromatics) and unsaturated chains (olefins) or rings (aromatics) are found in the oil component. At each process step, the oil and impurities content of the **wax(es)** is lowered. Materials similar to the oil component of the waxes are included in the Lubricating Oil Basestocks HPV Test Plan. While waxes are composed primarily of linear **alkane** molecules, the compounds in the Lubricating Basestocks category contain primarily of branched-chain alkanes and naphthenics.

Figure 1. Schematic of the Production Process for Waxes and Related Materials



A detailed description of petroleum refining, including the production of the waxes and related materials can be found in the OSHA Technical Manual (OSHA, 1999).

Based on the degree of processing they receive (and the corresponding reduction in oil content and impurities) and their physical properties, the eight substances in the “waxes and related materials” category can be divided into three sub-categories, as shown in Table 1:

Table 1. Category Members

Sub-category	CAS # ¹	Substance
Slack Waxes	64742-61-6	Slack wax (Petroleum)
Refined/finished Waxes		
(Paraffin)	8002-74-2	Paraffin Waxes and Hydrocarbon waxes
	64742-43-4	Paraffin waxes (petroleum), clay treated
	64742-5 1-4	Paraffin waxes (petroleum), hydrotreated
(Microcrystalline)	63231-60-7	Paraffin waxes and hydrocarbon waxes microcrystalline
	64742-42-3	Hydrocarbon waxes (petroleum), clay-treated microcrystalline
	64742-60-5	Hydrocarbon waxes (petroleum), hydrotreated microcrystalline
Petrolatum	8009-03-g	Petrolatum (Petroleum Jelly)

¹Appendix A contains complete CAS descriptions

Slack waxes are generally limited to industrial applications such as lubricants and specialty cleaning/preservative products. Consequently, human exposures are primarily through the dermal route. As can be seen from the process diagram, slack waxes may undergo additional processing to produce finished/refined waxes and petrolatum. Slack waxes derived from low viscosity oils contain predominantly normal paraffins. Heavier oil fractions yield slack waxes with increasing proportions of isoparaffins, cycloparaffins and alkylated aromatics in addition to the normal paraffins. Commonly, slack waxes are derived from solvent-refined vacuum distillates, in which case they contain a very low content of alkylated aromatic hydrocarbons.

The Testing Group has been unable to locate detailed compositional information on slack waxes, particularly information on their PNA content. This is not surprising, since as noted earlier, the petroleum waxes and related materials are typically defined by process history and product use specifications, not by detailed compositional information. However, API has collected compositional information on vacuum residuum, the refinery stream from which slack wax is derived (as represented by the top box in Figure 1). Information received from 8 companies and analysis of 2 toxicity test samples showed aromatic contents ranging from 34.7 to 65.0 wt % (API, 1983; API, 1987). As part of the process of converting vacuum residuum to slack wax, the aromatic content of the refinery stream is reduced via an extraction step. Thus, the aromatic content of vacuum residuum represents the “worst case” with regard to aromatic content of slack wax.

Refined/finished waxes have a large and varied number of industrial, commercial, and consumer product applications. They may be used in lubricants, wire cables, candles, petroleum jellies, cosmetics, and food/drug applications. Thus, human exposures may be via both the dermal and oral routes. Refined/finished waxes that are intended for food, food contact, cosmetic, pharmaceutical and related applications have to meet stringent purity requirements as described in the respective national and international legislations. These generally specify melting ranges, color, polycyclic aromatic hydrocarbon content and other impurity limits. For instance, in regard to the

polycyclic aromatic hydrocarbon content, the U.S. FDA has established ultraviolet absorbance limits that set an upper limit of 0.5 mg/kg for the total concentration of all extractable PAH-compounds in the wax sample tested (U.S. FDA, 2001; CONCAWE 1984). The refined waxes may be divided into two subgroups based on melting point, the paraffinic waxes (lower melting paraffin waxes) and the microcrystalline waxes (or high melt waxes). The former are obtained from processing light lube distillate while the latter are obtained from processing vacuum residue, or heavier lube distillate. Paraffin waxes consist mainly of normal alkanes, varying amounts of isoalkanes, cycloalkanes and a very low concentration of alkylated aromatic hydrocarbons. Microcrystalline waxes consist of substantial amounts of iso- and cycloalkanes, usually with a lesser amount of normal alkanes and trace amounts of alkylated aromatic hydrocarbons.

Food-grade petrolatum (petroleum jelly) is a mixture of highly refined higher-melting **paraffin** wax and typically, greater than 10% USP-grade white mineral oil (evaluated in the Lubricating Oil Basestocks HPV Test Program). Petrolatum consists mainly of branched and straight chain alkanes. Petrolatum has a large and varied number of industrial, commercial, and consumer product applications. Like refined/finished waxes, it may be used in lubricants, wire cables, candles, petroleum jellies, cosmetics, and food/drug applications. And, as with refined/finished waxes, human exposures may occur by both dermal and oral routes. When used in food, food contact, cosmetic, pharmaceutical and related applications, petrolatum has to meet stringent purity requirements as described in the respective national Pharmacopoeia and international legislations. These regulations generally specify melting ranges, color, polycyclic aromatic hydrocarbon content and other impurity limits. As with food-grade refined/finished waxes, in regard to the polycyclic aromatic hydrocarbon content, the U.S. FDA has established ultraviolet absorbance limits that set an upper limit of 0.5 mg/kg for the total concentration of all extractable PAH-compounds in the wax sample tested (U.S. FDA, 2001; CONCAWE 1984).

Table 2. summarizes the range of physical and chemical properties that characterize the 3 subcategories of waxes (Bennet, 1975; Kauffman et al. 1993; EWP, 1990).

Table 2. Physicochemical Properties of the Three Subcategories of Waxes				
	Oil content (%m/m)	Carbon number range	Melting point (°C)	Kinematic viscosity at 100°C (mm ² /s) ²
Slack wax	2 - 30	C-12 - C-85	43 - 63	3 - 30
Refined/finished waxes				
Paraffin Wax	<2.5	C-12 - C-75	42 - 74	3 - 10
Microcrystalline Wax	< 5	C-23 - C-85	60 - 95	10-30
Petrolatum	> 10 ¹	C- 12 - C-85	36 - 60	3 - 30

¹ USP-grade white mineral oil

²Kinematic viscosity is also expressed in Centistokes (cSt). 1mm²/s = 1 cSt

CATEGORY RATIONALE AND TEST MATERIAL DESCRIPTION

The Testing Group has used the following assumptions when analyzing the existing data, proposing testing and selecting a test material:

1. The materials included in the Waxes and Related Materials category are similar from both process and physical/chemical perspectives
2. Materials in the category are composed of varying ratios of two major components:
 1. Wax
 2. Oil

3. The physical state (semi-solid to solid) and the number of carbon atoms of the wax component severely limit its bioavailability and its environmental distribution, breakdown, or conversion. Toxicity data on refined/finished waxes and petrolatum can be used to characterize the wax component of all members of this category.
4. The potential toxicity of the materials included in the Waxes and Related Materials category is associated with the biologically available/active impurities such as **PAC/PNA** (polycyclic aromatic **compounds/polynuclear** aromatics) and unsaturated chains (olefins) or rings (aromatics).
5. These biologically available/active impurities are found in the oil component of the materials in the category, not in the wax component. Materials similar to the oil component of the materials in this Test Plan are included in the Lubricating Oil Basestocks HPV Test Plan. Because of this similarity, information from the Lubricating Oil Basestocks HPV Test Plan may be used by the Testing Group to augment information contained in the Waxes HPV Test Plan.
6. In the process of converting slack waxes to refined/finished waxes and then to food-grade **waxes/petrolatum**, oil containing potentially biologically available/active impurities is removed from the material. Consequently, the potential toxicity of the materials decreases as they are processed from slack wax to refined/finished waxes to food-grade waxes/petrolatum.

To supplement existing data on the various sub-categories of waxes, the Testing Group will conduct selected toxicity tests on a representative sample of slack wax. The Testing Group thinks the toxicity testing of slack wax will address the HPV data needs of all three subcategories of waxes found in this category. Of the three subcategories of waxes, slack wax is the least refined stream. It therefore contains the broadest spectrum of chemical components of all the categories of materials addressed in this category. The use of slack wax as a test sample will maximize any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

The Testing Group is planning to conduct the slack wax mammalian toxicity testing via the dermal route because:

1. The physical/chemical nature of slack wax (semi solid),
2. The primary route of human exposure to this material is dermal, and
3. Administration via the dermal route minimizes the potential “first pass” metabolism by the liver of the biologically available/active impurities.

The Testing Group thinks the test results obtained on slack wax via the dermal route of exposure can be “read across” to both dermal and oral routes of exposure for the refined/finished waxes and petrolatum because:

1. Historic data shows absorption of **PNAs** via both dermal and oral routes of exposure in the rat,
2. As noted above, administration via the dermal route minimizes the potential “first pass” metabolism by the liver of the biologically available/active impurities

Specific analytical data on the slack wax test sample will be available when the sample is obtained. The test sample will have **physicochemical** properties similar to those shown in Table 2. The Testing Group will attempt to select a test sample with a high oil and **PNA/impurities** content, thereby maximizing the sample's potential biological activity.

Coordination with Other Test Programs and Plans

To avoid duplication of effort and unnecessary use of animals, the Petroleum HPV Testing Group is coordinating its efforts with two of its other test plans. To this end, the Testing Group is relying on the Lubricating Oil Basestocks and Aromatic Extracts Test Plans to provide supplementary data for the oil and aromatic components of the materials in the waxes category.

EVALUATION OF EXISTING HEALTH EFFECTS DATA AND PROPOSED TESTING

General Evaluation

The Test Plan addresses the health effects endpoints of the category by:

1. Evaluating the extensive toxicology database for the three sub-categories of waxes (slack waxes, finished/refined waxes and petrolatum),
2. Using read-across information whenever possible among and between the sub-categories,
3. Maximizes any efforts to detect adverse effects by proposing to perform selected toxicity testing on slack wax, the category member that contains the broadest spectrum of and highest concentration of potentially bioavailable/biologically active components, and
4. Anticipating the availability of “read-across” information from the Lubricating Oil Basestocks HPV Test Plan to further characterize the oil component of the category members.

Toxicological data on waxes have been reviewed by an expert panel of the Cosmetics, Toiletries and Fragrances Association (CTFA, 1981), and the review was published in the Journal of the American College of Toxicology (Elder, 1984). The EU Scientific Committee for Food (SCF) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) also assessed the safety of waxes for use as food additives and as food contact materials. The outcome of their reviews and the oral allowable daily intakes (ADIs) that were established has been published (SCF, 1995; JECFA 1996).

Acute Toxicity

Slack waxes • There are no acute toxicity data available for the slack waxes. However, acute toxicity data does exist for both the more highly refined waxes and the base oils that contain the potentially biologically available/active portion of these materials. (Materials similar to the base oils are included in the Lubricating Oil Basestocks HPV Test Plan). Since these represent the two components of the slack waxes, the Testing Group thinks the existing information can be extrapolated to the slack waxes.

Refined/finished waxes • Oral LD₅₀ values greater than 5000 mg/kg have been found for samples of both paraffin and microcrystalline waxes (IBR, 1976 a & b). A 50/50% solution of petrolatum and a paraffin wax resulted in a dermal LD₅₀ > 4000 mg/kg and slight eye irritation. Paraffin and microcrystalline waxes have been reported to be non- and slightly irritating to the skin, respectively. The information on the non-oral endpoints is taken from a published safety review conducted by an expert panel (Elder, 1984). While few experimental details are provided and the quality of the studies and the panel’s conclusions cannot be verified, the Testing Group thinks the information is of sufficient quality to allow it to be used to fulfill the data needs for the acute toxicity endpoint.

Petrolatum • No acute oral toxicity data are available. The acute toxicity potential can be estimated from information on refined/finished waxes and the highly refined base oils used to produce these materials (included in the Lubricating Oil Basestocks HPV Test Plan).

A 50/50% solution of petrolatum and a paraffin wax resulted in a dermal LD₅₀ > 4000 mg/kg and slight eye irritation. The information is taken from a published safety review conducted by an expert panel (Elder, 1984). Whereas few experimental details are provided and the quality of the studies and the panel’s conclusions cannot be verified, the Testing Group thinks the information is of sufficient quality to allow it to be used to fulfill the data needs for the acute toxicity endpoint.

Because of their food grade application, a significant amount of acute, subchronic, chronic, reproductive, and sensitization testing has been done on white mineral oils, the oil component of petrolatum. These data are discussed in the HPV Lubricating Oil Basestocks HPV Test Plan.

Summary: No additional testing is planned. Multiple acute toxicity studies have been reported on the waxes and the base oil streams from which they are derived. Ancillary data is also available on the consumer products in which the refined/finished waxes and petrolatum are used, i.e. cosmetics. All these studies have consistently found these materials to have low acute toxicities. The Testing Group thinks the existing data is sufficient to characterize the acute toxicities of this category of materials.

Repeated Dose Toxicity

Slack waxes • Three skin carcinogenicity studies have been reported on slack wax. The quality of these three carcinogenicity studies on slack wax cannot be verified since the reports do not provide a complete set of experimental details. Only one of the studies tested a wax produced by solvent extraction, the current method of preparation. The other two reports are on waxes produced by the older process of “pressing” unfinished or poorly

finished materials. When compared to the waxes currently produced, the older “pressing” process resulted in waxes with higher levels of biologically available/active impurities such as **PAC/PNA** (polycyclic aromatic compounds/polynuclear aromatics) and unsaturated chains (**olefins**) or rings (aromatics).

The two slack waxes that were produced by the solvent extraction process currently used in refineries were not tumorigenic after application of 25 mg to the backs of male mice 2 days/week for 80 weeks (Kane et al., 1984).

Eight slack waxes (produced by the older process of “pressing”) and the corresponding aromatic extract¹ fractions were tested in a lifetime skin painting study (Smith et al., 1951). Approximately 15 mg of test material were applied 3 days/week to the backs of male albino mice (groups of $n=30$). At 250 days, the authors reported the slack waxes showed a low order of carcinogenicity, benign skin tumors were observed in 6 of the 8 groups and malignant skin tumors in 2 groups. At 450 days, benign skin tumors had developed in all groups, but malignant tumors had developed in only 5 of the 8 groups. The aromatic extracts exhibited a greater potency. At 250 days all but one sample had produced papillomas and 5 samples had produced cancers. At 450 days all had elicited benign tumors and all but one sample had elicited cancers. The authors concluded that the slack waxes were weakly carcinogenic and that carcinogenic activity was caused by the aromatic compounds found in the oils component, rather than the **paraffinic** waxes component. Similar results were produced by an additional study from the same laboratory on 11 slack waxes (again, produced by the older process of “pressing”) (Dietz et al., 1952).

Included in the Testing Group’s weight of evidence analysis was the fact that all three studies were reported in the peer review literature and had results consistent with not only each other, but numerous other dermal carcinogenicity studies done on petroleum hydrocarbons. The Testing Group thinks the weight of evidence provided by the aggregate results of these studies are adequate for the carcinogenic endpoints evaluated. These studies also demonstrate the dermal absorption of the biologically active compounds.

However, because the reports of these studies lack experimental details, the Testing Group has concluded the studies do not provide an adequate database for assessing the potential non-carcinogenic repeated-dose toxicity of slack wax.

Refined/finished waxes • A series of 90-day rodent feeding studies have been conducted on various white mineral oils (possible components of petrolatum) and food-grade low and high melting point refined/finished waxes (BIBRA, 1992; 1993; 1999). The waxes were incorporated in the animals’ diets at concentrations up to 2.0% by weight. Animals dosed with either low viscosity oils or a low melting point paraffin wax had evidence of inflammatory changes in the liver, spleen, and lymph nodes and **macrophage**. Higher molecular weight oils and microcrystalline waxes were without effect). In addition, low molecular weight (low-melting point) waxes unexpectedly produced heart mitral valve accumulation of wax/crystal material in a single strain of rat (Fischer 344), an effect not observed with any other test materials.

Recent, multiple lifetime chronic/cancer studies of medium and high molecular weight white oils confirmed the earlier wax and oil studies (These studies will be assessed in the Lubricating Oil Basestocks HPV Test Plan).

In an earlier study reported by Shubik et al. (1962) the toxicity of a variety of refined/finished waxes were investigated via the oral, dermal and subcutaneous routes of exposure. Five refined/finished wax samples were fed to male and female rats at a dietary concentration of 10% (approximately 5000 mg/kg/day) for a period of 2 years. There were neither treatment-related changes in survival or growth, nor any abnormal effects at necropsy or upon histological examination. Tumor incidence was unaffected by treatment. The chronic dermal exposure studies were conducted in both mice and rabbits. Five refined/finished waxes (15% concentration in benzene) were applied 3 times per week throughout the animals’ lifetimes. Treated groups showed mild irritation (limited to desquamation and depilation) that persisted throughout the study. However, there was no evidence of treatment-related tumor incidence or other effects. This study was not reported thoroughly, nor had it been completed at the time the paper was published. Due to the lack of experimental detail in the published report and the fact the study was done before the GLP guidelines were established, the Testing Group is unable to assess the reliability of the rabbit portion of the study.

¹ Aromatic extracts are products derived from the aromatic components extracted from the residual fractions from atmospheric & vacuum distillation that are then processed into slack waxes.

Finally, Shubik et al. (1962) implanted subcutaneously into groups of male and female mice disks made of 5 different refined/finished waxes. The Testing Group does not consider this to be a relevant route of exposure for human exposure. Consequently, the results from these studies are not discussed in this Test Plan.

Petrolatum - In an oral carcinogenicity study, 3 blends of pharmaceutical and food-grade petrolatums were fed to male and female rats at dietary concentrations of 5% for 2 years (Oser et al., 1965). No treatment-related changes were observed during treatment (body weight, blood chemistry, and hematological endpoints), at necropsy (weights of liver, spleen, kidney, heart, adrenals, thyroid, and pituitary), or through histological examination (a range of tissues). None of the three petrolatum blends produced an increase in tumor incidence.

The 1965 report by Oser et al. also included the results of a chronic study of petrolatum administered subcutaneously. The Testing Group does not consider this to be a relevant route of exposure for human exposure. Consequently, the results from these studies are not discussed in this Test Plan.

There are reports of two dermal carcinogenicity studies on petrolatum. In the first study, no tumors developed following application of two samples of petrolatum (25 mg twice weekly for 80 weeks) to male mice (Kane et al., 1984). The quality of this work cannot be verified since the report that includes these results is a summary of an extensive program of studies and does not include all the experimental details.

In the second study, 60 -I of a 15% solution of amber petrolatum (petrolatum NF grade) in iso-octane was applied twice weekly to the skin of male and female mice for 2 years. It was concluded that amber petrolatum was not carcinogenic (Li,jinsky et al., 1966).

Summary: Slack wax will be tested via the dermal route using a 2%day combined, repeated dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). The Testing Group thinks the existing chronic toxicity studies on slack waxes do not adequately address non-carcinogenic endpoints.

Reproductive Toxicity

No studies have been reported on the reproductive toxicity of petroleum waxes and related materials.

Summary: Slack wax will be tested via the dermal route using a 28day combined, repeated dose and reproductive/developmental toxicity screening protocol (OECD Test Guideline 422). The Testing Group thinks the testing of slack wax will address the hazards of all three sub-categories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components of all the categories of materials addressed in this program. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

Genotoxicity

No studies have been reported on the genotoxicity of petroleum waxes and related materials.

The Testing Group thinks the genotoxicity testing of slack wax will screen all three sub-categories of waxes found in this category. This is because slack wax, as the least processed of the materials, contains the broadest spectrum of chemical components of all the categories of materials addressed in this program. The use of slack wax as a test sample therefore maximizes any efforts to detect adverse effects. If any of the testing on slack wax produces significant toxicity, additional testing of the more highly refined/finished products may be undertaken.

While the Testing Group shares the desire to limit animal testing by using *in vitro* methodologies when possible, it decided to conduct the *in vivo* micronucleus test for the following reasons:

1. The physical/chemical nature of the test material precludes testing the intact material *in vitro*.
2. It could be performed using animals that were already included in the repeat dose 28-day study, and
3. It eliminates the need to perform an additional study solely for the purpose of studying *in vivo* genotoxicity.

Summary: Slack wax will be tested in the *in vitro* bacterial reverse mutation assay (OECD 471) and the *in vivo* mammalian erythrocyte micronucleus test (OECD 474). The *in vivo* micronucleus test will be included in the **28-day** repeat dose study on slack wax (see “Repeated Dose Toxicity” section).

Human Experience

In addition to the animal studies summarized above, clinical studies and human experience with the use of materials in this category are also available. The information is consistent with the published animal toxicology studies and the program of testing recommended in this test plan.

Slack wax • There have been several reports of cancer in wax pressmen exposed to unfinished /poorly finished oil during the preparation of paraffin wax (Hendricks et al., 1959; Lione and Denholm, 1959). Due to the refining processes at the time, these oils contained high concentrations of polycyclic aromatic hydrocarbons. However, current refining processes are much more stringent and consequently the oils contain much lower levels of polycyclic aromatic hydrocarbons in the oil,

Refined/finished wax • Clinical studies with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16%) and microcrystalline (4.35-15%) waxes were reviewed (Elder, 1984). These studies included a range of acute and repeat application tests in groups of humans to observe skin irritation and skin sensitization potentials. All products produced, at most, slight erythema, and none caused skin sensitization.

The widespread use of paraffin wax in cosmetics and in cosmetic surgery over many years demonstrates the low toxicity of finished waxes and the many guidelines for their safe use (Hjorth, 1987). There have been few reports of adverse effects, such as subcutaneous deposits following injection under the skin (often referred to as paraffinoma), but these are not normally associated with progressive changes (Ho et al., 2001). Another report described an outbreak of skin rashes attributed to occupational exposure to wax fumes (Halton and Piersol, 1994).

Petrolatum • Despite the widespread use of petrolatum for many years as a vehicle in human skin patch testing, only isolated cases of allergy to petrolatum have been reported (Frankel, 1965; Dooms-Goosens and Degreef, 1983; Ayadi and Martin, 1987; Fisher, 1981; Conti et al., 1995).

EVALUATION OF PHYSICOCHEMICAL AND ENVIRONMENTAL FATE DATA AND PROPOSED TESTING

General Evaluation

Based on the measured and predicted behavior of the constituent hydrocarbons these substances are nonvolatile materials, do not contain any oxidizing constituents, and are almost totally insoluble in water. Therefore the hydrocarbon components of these substances will have little or no tendency to partition to air, are not susceptible to hydrolysis or direct photolysis under environmental conditions, and will partition primarily to soil and sediment.

Physicochemical Data

Measured data for specific physicochemical properties of the representative substances in the waxes category that can be used in the HPV chemicals program were not available. There are estimation structure-activity relationships for the physicochemical endpoints in the computer program EPIWIN (Estimation Program Interface for Windows) and EPA has suggested that subroutines in this program would be acceptable to develop data for these endpoints. Because of the diversity of compounds encompassing waxes, it is not feasible to model the physicochemical endpoints for each potential compound. Rather, modeling efforts were directed towards those compounds of the waxes that would most likely be dispersed to various environmental media. Since molecular weight and structural conformation determine in large part the solubility and vapor pressure characteristics of the hydrocarbons, modeling focused on paraffinic, naphthenic, and aromatic compounds containing thirteen carbon atoms. The C13 compounds are among the shorter chain-length molecules present in waxes and comprise only a small fraction of most waxes.

Melting Point

Adequate data exist.

Boiling Point

Adequate data exist.

Vapor Pressure

Adequate data exist.

Partition Coefficient (Log K_{ow})

The percent distribution of the hydrocarbon groups (i.e., paraffins, naphthenes, and aromatics) and the carbon chain lengths determines in part the partitioning characteristics of the mixture. Generally, hydrocarbon chains with fewer carbon atoms tend to have lower partition coefficients than those with higher carbon numbers (CONCAWE, 2001). However, due to their complex composition, unequivocal determination of the log K_{ow} cannot be made. Rather, partition coefficients of selected C13 chain length hydrocarbon structures representing paraffinic, naphthenic, and aromatic constituents in base oil lubricants were modeled using the **EPIWIN[®]**, **WSKOW V1.40** computer model (U.S. EPA, 2000). Results showed typical log K_{ow} values from 4.9 and higher, which was consistent with values of **>4** for lubricating oil base-stocks reported by CONCAWE (1997).

Summary: No additional modeling is proposed. Partition coefficients (K_{ow}) have been calculated for representative components of C 13 hydrocarbon structures.

Water Solubility

As noted for partition coefficient, the water solubility of waxes cannot be determined due to their complex mixture characteristics. Therefore, the water solubilities of individual C13 hydrocarbons representing the different groups making up waxes (i.e., linear and branched paraffins, naphthenes, and aromatics) were modeled using **WSKOW V1.40**. Based on water solubility modeling of those groups (typically much less than 1 ppm), waxes should be considered almost totally insoluble in water.

Summary: No additional modeling is proposed. Water solubility values have been calculated for representative wax components.

Environmental Fate Data

The typical battery of tests used to measure the environmental fate of a material is not easily performed on the materials of this category because of their physical and chemical properties.

Photodegradation

Chemicals having potential to photolyze have **UV/visible** absorption maxima in the range of 290 to 800 nm. The hydrocarbon constituents in this category are not expected to photolyze since they do not show absorbance within the 290-800 nm range.

Although wax components typically have low vapor pressures some lower molecular weight components (e.g., C13 branched paraffins and naphthenes) may volatilize, thus creating the potential for atmospheric oxidation. However, these lower molecular weight compounds exhibit atmospheric oxidation half-lives of less than one day (12 hours). Therefore, those compounds that may partition to the atmosphere will be removed through indirect photochemical degradation. Calculation of atmospheric oxidation potential (AOP) was applied to specific hydrocarbons in this category in order to estimate the range of vapor-phase reactivity. The AOP was determined using the **EPIWIN[®]** model, **AopWIN V1.90** (U.S. EPA, 2000).

Summary: No additional modeling is proposed. Atmospheric half-lives have been calculated for various C 13 paraffins, naphthenes and aromatics.

Stability in Water

Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Materials in the waxes category are not subject to hydrolysis because they lack functional groups that hydrolyze.

Summary: Computer modeling will not be conducted for materials in the waxes category because they do not undergo hydrolysis.

Chemical Transport and Distribution in the Environment (Fugacity Modeling)

The US EPA has agreed that computer-modeling techniques are an appropriate approach to estimating chemical partitioning (fugacity is a calculated endpoint and is not measured). A widely used fugacity model is the EQC (Equilibrium Criterion) model. The EQC model is a Level 1 (i.e., steady state, equilibrium, closed system and no degradation) model that utilizes the input of basic chemical properties including molecular weight, vapor pressure, and water solubility to calculate distribution within a standardized regional environment. EPA cites the use of this model in its document titled, "Determining the Adequacy of Existing Data, which was prepared as guidance for the I-IPV chemicals program." The model was used to estimate the percent distribution in environmental media of various C13 to C29 compounds representing the different classes of hydrocarbons found in waxes.

Summary: No further modeling is proposed. Fugacity modeling has been done to provide an estimate of the percent distribution in environmental media of various C13 to C29 compounds representing selected hydrocarbon classes found in waxes.

Biodegradation

The assimilation of paraffin waxes has been described for a diverse range of microorganisms in laboratory studies. A wide range of bacteria, yeasts and fungi grows on paraffin wax as a sole carbon source under aerobic conditions (Rahn, 1906; Sohngen, 1913; Fuhs, 1961; Miyamoto, 1968). Initial decomposition of wax is similar to the degradation of short chain alkanes (Miyamoto, 1968). Assimilation of a highly finished paraffin wax containing 91% normal paraffins from C-25 to C-37 (mainly C28 to C32) occurs in several species of bacteria and yeast (Yamada and Yogo, 1970). Also, yeast cultures grown on paraffin wax results in typical cell yields of 70%, although the paraffin wax is not completely utilized (Miller and Johnson, 1966).

Inherent biodegradability studies have been performed on samples of paraffin wax (CAS No 8002-74-2), intermediate wax (CAS No 97489-05-9) and microcrystalline wax (CAS No 63231-60-7) using a shake-flask procedure (referred to by the authors as OECD 301B Modified Sturm test) in which the materials were exposed on glass fiber filters. In these tests, the paraffin wax (CAS no. 8002-74-2) degraded by 81% after 28 days and by 87% after 84 days, the intermediate wax (CAS no. 97489-05-9) degraded by 66% after 28 days and by 77% after 84 days and the microcrystalline wax (CAS no. 63231-60-7) degraded by 21% after 28 days and by 25% after 84 days. Member company data using paraffin and microcrystalline waxes in a shake-flask test using un-acclimated inocula from sewage sludge and soil generally corroborate the mineralization data from this study. In those tests (see Robust Summary for details), the paraffin wax mineralized by 55% in 31 days (comparable to 28-day data) and by 98.5% in 137 days. Comparison of the results of the two studies (Hanstveit, et al 1989 and company data) for paraffin waxes indicates both that microbial communities may well become enriched in capable species during an acclimation period, based on the day 28 inherent versus day 31 ready biodegradation results (81% versus 55%), and that the hydrocarbons remaining after the standard incubation period continue to be mineralized, based on the day 84 and day 137 results. Conversely, the microcrystalline wax results indicate that acclimation does not tend to enhance the degree of biodegradation of these more-complex materials. In particular, the presence of the higher molecular weight hydrocarbons seems to be a limiting factor, in that they are not biologically available for metabolism. The extended incubation results obtained in the company studies of microcrystalline wax (27% mineralization after 31 days, and 67% after 137 days) indicate some potential for increased degradation over longer periods, contrary to the other tests using this material. This may arise as a result of different enzymatic capabilities of the inocula, or as an artifact of the extended incubation time. In litter bag tests performed on the same three samples as were used in the inherent biodegradability studies, paraffin and intermediate waxes were 100% degraded after three months exposure in woodland soil in autumn/winter. Under the same conditions, microcrystalline waxes were only 20% degraded after 6 months exposure, expressing the slower rate of biodegradation of these substances. Battersby et al (1992) reported 28-day ready biodegradability studies on three samples of hydrotreated slack wax (CAS No. 92062-09-4) of different viscosities using the modified Sturm method (OECD guideline 301B). All the samples were emulsified in the test medium using a hard anionic surfactant. The observed biodegradabilities were 26%, 41% and 48% indicating that these substances are inherently biodegradable but not readily biodegradable in water.

An environmental study was conducted on the decomposition of paraffin, intermediate, and microcrystalline waxed paper samples in a woodland leaf litter layer (Hanstveit, 1991b). During a six-month winter period, degradation of paraffin and intermediate waxed paper samples occurred almost completely in 5 mm mesh bags with a half-life of approximately two months. Lower decomposition rates occurred in the summer period and for the microcrystalline

waxed paper sample. Both soil micro- and macro-organisms contributed to the decomposition under field conditions.

It may be concluded that paraffin waxes are inherently biodegradable, while microcrystalline waxes contain hydrocarbons that are more resistant to biodegradation.

Summary: No additional testing is proposed. Sufficient data exists to characterize the biodegradability of these waxes and related materials.

EVALUATION OF ECOTOXICITY DATA AND PROPOSED TESTING

There is no published information on the aquatic toxicity of petroleum waxes. However, work by Adema and van den Bos Bakker (1986) on the ecotoxicity of alkanes to *Daphnia magna*, *Chaetogamarus marinus* and *Mysidopsis bahia* shows that alkanes of carbon number greater than C-10 are not acutely toxic to these species at their limit of solubility in water. Because paraffin waxes are largely composed of alkanes of carbon number greater than C-20, they are not expected to cause acute toxicity to aquatic invertebrates. The results of toxicity tests with lubricant base oils, which have similar hydrocarbon ranges and some structures in common, show no acute toxicity to freshwater fish, invertebrates, or algae and no chronic effects to aquatic life at concentrations below 1 mg/L (CONCAWE, 1997). Some bioaccumulation of lower molecular weight components from the oils/waxes would be expected to occur to a limited degree based on animal and environmental studies (CONCAWE, 1997). However, work by Parkerton et. al. (2001) has shown that those hydrocarbon substances with calculated octanol-water partition coefficient (log P) values ≥ 3 indicating a high potential to bioaccumulate, were readily metabolized based on fish tissue analysis from dietary bioaccumulation studies. Higher molecular weight hydrocarbons (C12 and up) with structural linearity showed faster depuration rates than the branched isomers. Therefore, although log P values may indicate potential to partition to fat/tissue compartments in aquatic organisms, bioaccumulation does not occur in all instances due to metabolism. Similarly, by inference from the results of chronic ecotoxicity studies conducted on lubricating oil basestocks (CONCAWE, 1997), wax and petrolatum are not expected to cause chronic effects in aquatic organisms.

In February of 2001 discharge of slack wax to national parks along British Columbia (Canada) coastline occurred during tank washing activities, impacting approximately 100 km of beaches in the Pacific Rim National Park. Canadian Wildlife Service (a branch of Environment Canada) and the Department Of Fisheries And Oceans biologists agreed that the risk of acute toxicity to aquatic life in the area was minimal based on the low solubility of the components in the wax and given that the BC parks staff observed no significant environmental impacts. Generally the consensus was that the material was relatively inert and would likely pose little environmental damage (ExxonMobil Biomedical Sciences Inc., 2001).

Summary: No additional tests are proposed.

MATRIX OF AVAILABLE DATA AND PROPOSED TESTING

Table 3: Waxes and Related Materials: Matrix of Available Data and Proposed Testing

Test	Slack Wax	Petrolatum	Refined Wax
Physical/Chemical Properties			
Melting Point	Adequate	Adequate	Adequate
Boiling Point	Adequate	Adequate	Adequate
Vapor Pressure	Adequate	Adequate	Adequate
Water Solubility	Adequate	Adequate	Adequate
Partition coefficient (log Kow)	Adequate	Adequate	Adequate
Ecotoxicity			
Algae Growth Inhibition	Adequate	Adequate	Adequate
Acute Freshwater Invertebrate	Adequate	Adequate	Adequate
Acute Freshwater Fish	Adequate	Adequate	Adequate
Environmental Fate			
Biodegradation	Adequate	Adequate	Adequate
Stability in Water	Adequate	Adequate	Adequate
Photodegradation (estimate)	Adequate	Adequate	Adequate
Transport and Distribution	Adequate	Adequate	Adequate
Mammalian Toxicity			
Acute	Read-Across ¹	Adequate	Adequate
Repeat Dose	TEST	Adequate	Adequate
Repro/Develop	TEST	Read-Across ²	Read-Across ²
Genotoxicity, <i>in vitro</i> & <i>in vivo</i>	TEST	Read-Across ²	Read-Across ²
¹ Data will be read across from the petrolatum & refined waxes subcategories; and the Lubricating Oil Basestocks Test Plan			
Data will be read across from the slack wax subcategory			

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APPENDIX A.

CAS Descriptions of Category Members

The CAS descriptions for refinery streams, including the petroleum waxes, were intentionally written to be qualitative in nature. Section 8(b) of the Toxic Substances Control Act required identification and registration with the Environmental Protection Agency before July 1979 of each “chemical substance” being manufactured, processed, imported or distributed in commerce. Due to analytical limitations, identification of every specific individual molecular compound in every refinery process stream under all processing conditions was impossible. In addition, there is known variability in stream composition due to things such as the crude oil used and small changes in process conditions. Even with reference to TSCA’s Candidate Inventory List, members of the industry would have reported refinery streams using a wide variety of names, with each company’s differing from all others. Perhaps 3000 or more different names for refinery streams would have been submitted to the EPA by the petroleum industry. Recognizing these problems, in 1977 API initiated an effort to compile a list of terms consistent with industry operations and with nomenclature included in API’s Thesaurus of petroleum industry technical terms.

As a result of this effort, API recommended to the EPA a list of generic names for refinery streams covering all known processes used by refiners. A definition of each stream was included, giving typical carbon number distribution, boiling or viscosity range, or general composition. Along with CAS numbers, this information was published by EPA as “Addendum I, Generic Terms Covering Petroleum Refinery Process Streams.”

As can be seen from the listing below, the descriptions accompanying the CAS number of each petroleum wax are written in broad, general terms. The descriptions often contain ranges of values, with little if any quantitative analytical information or concern for possible compositional overlaps. In these descriptions, process history, specifically the final process step, and not chemical composition, was one of the primary criteria to differentiate streams and assign CAS numbers. As a result, streams with the same or substantially similar compositions may have different CAS numbers if they originate in different process units.

CAS Number

64742-61-6

Slack wax, petroleum

A complex combination of hydrocarbons obtained from a petroleum fraction by solvent crystallization (solvent dewaxing) or as a distillation fraction from a very waxy crude. It consists predominantly of saturated straight and branched chain hydrocarbons having carbon numbers predominantly greater than C20.

8002-74-2

Paraffin waxes and Hydrocarbon waxes

A complex combination of hydrocarbons obtained from petroleum fractions by solvent crystallization (solvent deoiling) or by the sweating process. It consists predominantly of straight chain hydrocarbons having carbon numbers predominantly greater than C20.

64742-43-4

Paraffin waxes, petroleum, clay-treated

A complex combination of hydrocarbons obtained by treatment of a petroleum wax fraction with natural or modified clay in either a contacting or percolation process to remove the trace amounts of polar compounds and impurities present. It consists predominantly of straight chain saturated hydrocarbons having carbon numbers in the range of C20 through C50.

64742-5 1-4

Paraffin waxes, petroleum, hydrotreated

A complex combination of hydrocarbons obtained by treating a petroleum wax with hydrogen in the presence of a catalyst. It consists predominantly of straight chain paraffinic hydrocarbons having carbon numbers predominantly in the range of about C20 through C50.

63231-60-7

Paraffin waxes and Hydrocarbon waxes, microcryst.

A complex combination of long, branched chain hydrocarbons obtained from residual oils by solvent crystallization. It consists predominantly of saturated straight and branched chain hydrocarbons predominantly greater than C35.

64742-42-3

Hydrocarbon waxes, petroleum, clay-treated microcryst.

A complex combination of hydrocarbons obtained by treatment of a petroleum microcrystalline wax fraction with natural or modified clay in either a contacting or percolation process to remove the trace amounts of polar compounds and impurities present. It consists predominantly of long branched chain hydrocarbons having carbon numbers predominantly in the range of C25 through C50.

64742-60-5

Hydrocarbon waxes, petroleum, hydrotreated microcryst.

A complex combination of hydrocarbons obtained by treating a petroleum microcrystalline wax with hydrogen in the presence of a catalyst. It consists predominantly of long, branched chain hydrocarbons having carbon numbers predominantly in the range of C25 through C50.

8009-03-8

Petrolatum

A complex combination of hydrocarbons obtained as a semi-solid from **dewaxing** paraffinic residual oil. It consists predominantly of saturated crystalline and liquid hydrocarbons having carbon numbers predominantly greater than C25.

APPENDIX B.

Robust Summary (Separate Document)

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Appendix B.

ROBUST SUMMARY
OF INFORMATION ON

Substance Group:

WAXES
AND
Related materials

Summary prepared by: American Petroleum Institute

Creation date: SEPTEMBER 19, 2000

Printing date: AUGUST 6, 2002

Date of last Update: JULY 23, 2002

Number of Pages: 48

NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch, et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)

A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Solid
: This robust summary covers the waxes and related products which includes:
Slack wax
Petrolatum
Paraffin wax
Microcrystalline wax

Petroleum waxes are obtained from paraffinic refinery streams in lubricating oil manufacture.
The wax is separated by filtering a chilled solution of waxy oil in a selected solvent (usually a mixture of methyl ethyl ketone and toluene).

SLACK WAX is obtained from the dewaxing of refined or unrefined vacuum distillate fractions. If the material has been separated from residual oil fractions it is frequently called PETROLATUM.

The slack waxes are de-oiled by solvent crystallization or "sweating" processes to manufacture commercial waxes with low oil content. The oil that is separated from these processes is known as FOOTS OIL.

The refined petroleum waxes are known as PARAFFIN WAXES. MICROCRYSTALLINE WAXES have higher molecular weights than the paraffin waxes and consist of substantial amounts of iso- and cycloalkanes.

1.2 SYNONYMS

: Paraffin wax
Slack wax
Petrolatum
Microcrystalline wax

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 2 mg/m³
Remark : The UK HSE have established an occupational exposure limit of 2 mg/m³ (8 hour TWA) and a 15 minute Short Term Exposure Limit (STEL) of 6 mg/m³.

(2) (47)

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1.17 REVIEWS

**Memo
Remark**

- : EU SCF
 : The EU Scientific Committee for Food (SCF) reviewed the available information on mineral hydrocarbons, which included the petroleum waxes. Their opinion was published in 1995. The SCF reached the following conclusion:

There are sufficient data to allow a full Group ADI of 0-20 mg/kg bw for waxes conforming to the following specification:-

Highly refined waxes derived from petroleum based or synthetic hydrocarbon feedstocks, with

viscosity	not less than 11 mm ² /s (cSt) at 100° C
Carbon number	not less than 25 at the 5% boiling point
Average molecular weight	not less than 500

(43)

**Memo
Remark**

- : WHO JECFA
 : The WHO Joint Expert Committee on Food Additives (JECFA) reviewed the available information on food grade mineral hydrocarbons. Their evaluation was published in 1996. With respect to waxes they made the following conclusions:

Substance**ADI****(mk/kg bw)****Paraffin waxes**

LMPW (Low melting point wax)

ADI withdrawn

IMPW (Intermediate melting point wax)

ADI withdrawn

Microcrystalline waxes

HSW (High sulfur wax)

0-20

HMPW (High melting point wax)

0-20

(33)

**Memo
Remark**

- : CTFA
 : An independent expert panel reviewed data supplied to them by the Cosmetics, Toiletries & Fragrances Association (CTFA). A report of the evaluation was published in 1984. However, few experimental details are available and the conclusions of the panel cannot be verified. Their overall conclusion was:

Toxicological test data on Ozokerite, Ceresin, Montan Wax, Paraffin, Microcrystalline Wax, Emulsifying Wax N.F., and Synthetic Beeswax are presented. Based on the documented animal and clinical test data, it is concluded that these waxes are safe for use as cosmetic ingredients in the present practices of concentration and use.

(18)

2.1 MELTING POINT

Value : 36 - 95° C
: See additional remarks section 2.12

2.2 BOILING POINT

Value : ca. 350 - 500° C
: In a survey of the composition of food grade waxes and oils the boiling range for paraffin wax was reported to be 350-485°C. Microcrystalline waxes boiled in excess of 500 °C.

(11)

2.3.1 GRANULOMETRY

: Not relevant

2.5 PARTITION COEFFICIENT

Log Pow : 4.7 - \geq 6.7
Method : Calculated: KOWWIN Version 1.65 (EPIWIN)
Year : 2001
Test substance : Wax and related materials
Remark : As hydrocarbon number increases above C13, as is the case for the majority of the wax constituents, Log P values >6 are predicted. Substances having Log P estimates greater than 6 are characterized by extremely large molecular weight and subsequent hydrophobicity, therefore no significant aqueous exposures or bioaccumulation are expected to occur.
Result : Octanol-water partition coefficients (log P or Kow) were modeled with isomers of the lowest molecular weight component (C13 hydrocarbons) in waxes. These partitioning estimates are characteristic of only a small fraction of component molecules in a given wax. Because of the diversity of compounds encompassing waxes, it is not feasible to model the physicochemical endpoints for each potential compound. Since molecular weight and structural conformation determines in large part the solubility and vapor pressure characteristics of the hydrocarbons, modeling focused on the lower molecular weight hydrocarbons. These would be selected C13 and C20 hydrocarbons since waxes consist mostly of C20 to C85 compounds, with some minimal percent of C13 through C20 hydrocarbons. Therefore, the majority of the physicochemical modeling was performed on various paraffinic, naphthenic and aromatic representatives containing 13 and C20 carbon atoms.
Reliability : (2) valid with restrictions

(41)

2.6.1 WATER SOLUBILITY

Value : 0.027 - 5.96 mg/l at 25° C
Method : WSKOW Version 1.36 (EPIWIN)
Year : 2001
Test substance : Wax and related materials
Result : The water solubility of waxes cannot be determined due to their complex mixture characteristics. Therefore, the water solubility of individual C13 hydrocarbons was modeled. The highest solubilities would be exhibited by only a small fraction of the hydrocarbon molecules present in waxes. Increasing carbon number results in rapidly decreasing solubility, so that the most-soluble (predominantly methyl-substituted diaromatic) C18 and C20 analogues yield model values of 0.01195 and 0.00125 mg/l, respectively. Higher molecular weight (higher carbon number) components are even less water-soluble. Based on water solubility modeling for C13 components of complex mixtures, aqueous solubilities of these waxes are typically much less than 1 ppm, due to differential partitioning of components between the aqueous and organic phases.
Reliability : (2) valid with restrictions

(13)

2.8 AUTO FLAMMABILITY

: Not relevant

2.9 FLAMMABILITY

: Non flammable

2.10 EXPLOSIVE PROPERTIES

: Not relevant

2.11 OXIDIZING PROPERTIES

: Not relevant

2.12 ADDITIONAL REMARKS

:

Physico chemical properties for typical grades of wax and petrolatum are shown in the following table.

See also Bennet (1975), Kauffman et al (1993) and EWF (1990).

Melting Point (°C)	Kinematic Penetration viscosity at 100 °C	Oil content (%m/m) (mm ² /sec)	Carbon number range	(25°C)
ASTM D127	ASTM D445	ASTM D721 or D3235	ASTM D2505	ASTM D1321 or D937*
<u>Slack wax</u> 45-85	3-30	2-30	12-85	9-80*
<u>Lower Melt Paraffin Wax</u> 43-74	3-10	<2.5	18-75	9-50*
<u>Microcrystalline Wax</u> 60-95	10-30	<5	23-85	3-60*
<u>Petrolatum</u> 36-60	3-30	>10	12-85	>60

NB * The second value given for penetration was determined using method D937

(6) (20) (35)

3.1.1 PHOTODEGRADATION

Type : Atmospheric oxidation
Method : Calculated: AOPWin Version 1.89 (EPIWIN)
Year : 2001
Test substance : Wax and related materials
Remark : Although waxes typically have low vapor pressures, volatilization of some lower molecular weight components exhibit relatively high atmospheric oxidation half-lives. Therefore, those compounds that may partition to the atmosphere will be removed through indirect photochemical degradation. All modeled components exhibited rapid degradation in the atmosphere; the value presented represents both the most volatile component and the longest modeled half-life. All other modeled C13 components had both lower volatility and shorter half-lives.
Result : $T_{1/2} = 0.913$ days (10.96 hr) for most volatile C13 component modeled
Reliability : (2) valid with restrictions

(40)

3.1.2 STABILITY IN WATER

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. Materials in the waxes category are not subject to hydrolysis, as they lack these reactive groups.
Reliability : (1) valid without restriction

(28)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : Calculated according to Mackay Level I
Media : Soil, air, water, suspended sediment, and sediment
Year : 2000
Remark : Fugacity-based computer modeling indicated that the majority of high molecular weight hydrocarbons with carbon numbers of C20 and greater in waxes would be distributed to soil. Percent distribution estimates were modeled with C13 to C29 branched paraffins as this class of wax hydrocarbons shows the greater distribution to air. Aromatic compounds with carbon numbers from C13 through C85 will partition principally to soil. Linear paraffins and naphthenes distribute to both soil and air, with increasing partitioning to soil for hydrocarbons greater than C20 as vapor pressure decreases. Physical properties input are those calculated by the EPIWIN Estimation 3.04 program and included in this summary. Since the majority of hydrocarbon components in waxes are primarily normal paraffins of C20 and greater, with moderate to minimal amounts of naphthenics, isoparaffins and trace amounts of aromatics, volatility is not a significant fate process for these petroleum substances due to negligible vapor pressures at ambient temperatures and their high molecular weight. As hydrocarbon number increases above C20, partitioning to soil is the

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predominant behavior of these compounds.

Result	Carbon No. Isoparaffin	Air	Soil	% Distribution		Susp. Sediment	Biota
				Water	Sediment		
	C13	98	1.9	7E-3	4E-2	8E-3	1E-4
	C18	69	30	4E-4	0.68	2E-2	2E-3
	C20	33	65	2E-5	1.4	3E-2	4E-3
	C21	18	80	5E-6	1.8	5E-2	4E-3
	C22	12	86	2E-6	1.9	6E-2	4E-3
	C24	6	92	2E-7	2.1	6E-2	5E-3
	C26	1	97	2E-8	2.1	7E-2	5E-3
	C29	0.1	98	9E-10	2.2	7E-2	6E-3
Reliability	: (2) valid with restrictions						

(38)

3.5 BIODEGRADATION

Type	: Aerobic
Inoculum	: Oil-contaminated soil from land-farming project
Contact time	: 28 - 84 day
Result	: 80% in 28 days; inherently and extensively biodegradable
Deg. Product	: No
Method	: Modified OECD 301B (significant modification, actually shake flask test)
Year	: 1989
GLP	: Yes
Test substance	: Paraffin wax CAS 8002-74-2
Remark	: Paraffin wax residue analysis showed less than 10% parent hydrocarbons and some hydrocarbon enrichment from contaminated soil inoculum after 28 days.

Result	: <u>Degradation % after time</u>	80% of ThCO ₂ after 28 days;
		87% after 84 days (paraffins)
		66% of ThCO ₂ after 28 days;
		77% after 84 days (intermediate wax)

Kinetic (for sample, positive and negative controls)

Reference (sodium acetate) - Not Reported

Test substance - 80% (paraffin, 28 days),

66% (intermediate wax, 28days)

Test condition	<u>Breakdown Products</u>	No other than residual HCs
	: <u>Inoculum</u> : Soil was collected from land-farm used by the investigators to treat oil-contaminated soil. Soil contained 2200 mg/kg mineral oil (generally at greater retention times than wax components, based on chromatograms provided in report), and was a sandy loam comprising 68% sand, 14.2% clay and 10.2% silt with 5.4% OC. Elevated levels of heavy metals were measured in the soil but not considered to be inhibitory to the test. Soil was suspended in mineral medium prior to distribution to test vessels at a loading rate of approximately 80 mg/l. No microbial enumeration was undertaken but performance of the inoculum in degrading a reference standard (sodium acetate at 100 mg/l) provided evidence of inoculum adequacy.	

Concentration of test chemical: Test substance loading was approximately 20 mg/l of medium.

Temp of incubation: 20 \pm 2°C

Dosing procedure: Each 2-liter vessel contained 1 liter of inoculated medium. The wax was dissolved in heated carbon tetrachloride, then the solution applied to glass fiber filters (13 mm) to obtain about 20 mg wax/filter after evaporation of the solvent. One filter was added to each test material vessel. Controls and reference standards also received glass fiber filters to which CCl₄ was added and allowed to evaporate.

Sampling frequency: Carbon dioxide production was monitored weekly through day 28, and then every other week to day 84. Wax residues were measured only at test termination.

Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium acetate was used as the positive control.

Analytical method: Carbon dioxide production was measured by titrating residual base with 0.1 N HCl. Wax residues were measured by extracting filters with warm heptane and the volume of extract adjusted prior to GC-FID analysis.

Method of calculating biodegradation: Wax was assumed to have a mean composition of [CH₂] for the purpose of calculating ThCO₂ (3.14 mg CO₂/mg wax). The report does not include the mechanics of calculation of the mineralization endpoint. Total hydrocarbon remaining at 84 days was determined by area integration of the chromatograms, and primary biodegradability was determined by comparing the amount of hydrocarbons at the end of the test with the amount on wax-dosed filters prepared at the start of the test.

Other: Two grades of paraffin wax, 52/50 and 58/60 were tested; only the 52/50 grade was tested for 84 days, and in all, three tests were carried out for 52/50. Result below for 28 days is mean of 52/50 average and 58/60 result. An intermediate wax was also tested as noted in results.

Test substance was incubated in the inoculated mineral medium in sealed vessels containing a vial of 0.4 M NaOH (5 ml) suspended in the headspace above the medium (similar to EPA 835-3100). Carbon dioxide evolution resulting from mineralization of the test substance was trapped in the base for periodic quantitation. Base was renewed at each sampling period. GC analysis for parent compound was carried out on the solid phase of the test medium at study termination.

Reliability

: (2) valid with restrictions

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Type : Aerobic
Inoculum : Oil-contaminated soil from land-farming project
Contact time : 28 - 84 day
Result : Inherently biodegradable
Method : Modified OECD 301B (significant modification)
Year : 1989
GLP : Yes
Test substance : Microcrystalline wax CAS 63231-60-7
Remark : Wax residue analysis showed 65% parent hydrocarbons (mostly n-alkanes greater than C43) remained after 84 days. Most iso-alkanes were degraded regardless of carbon number.
Result : Degradation % after time: 21% of ThCO₂ after 28 days;
25% after 84 days

Kinetic (for sample, positive and negative controls:

Reference (sodium acetate) - Not Reported
Test substance - 21% (28d)

Breakdown Products: None

Test condition : Inoculum: Soil was collected from land-farm used by the investigators to treat oil-contaminated soil. Soil contained 2200 mg/kg mineral oil (generally at greater retention times than wax components, based on chromatograms provided in report), and was a sandy loam comprising 68% sand, 14.2% clay and 10.2% silt with 5.4% OC. Elevated levels of heavy metals were measured in the soil but not considered to be inhibitory to the test. Soil was suspended in mineral medium prior to distribution to test vessels at a loading rate of approximately 80 mg/l. No microbial enumeration was undertaken but performance of the inoculum in degrading a reference standard (sodium acetate at 100 mg/l) provided evidence of inoculum adequacy.

Concentration of test chemical: Test substance loading was approximately 20 mg/l of medium.

Temp of incubation: 20 ±2°C

Dosing procedure: Each 2-liter vessel contained 1 liter of inoculated medium. The wax was dissolved in heated carbon tetrachloride, then the solution applied to glass fiber filters (13 mm) to obtain about 20 mg wax/filter after evaporation of the solvent. One filter was added to each test material vessel. Controls and reference standards also received glass fiber filters to which CCl₄ was added and allowed to evaporate.

Sampling frequency: Carbon dioxide production was monitored weekly through day 28, then every other week through day 84. Wax residues were measured at test termination.

Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Sodium acetate was used as the positive control.

Analytical method: Carbon dioxide production was measured by titrating

residual base with 0.1 N HCl. Wax residues were measured by extracting filters with warm heptane and the volume of extract adjusted prior to GC-FID analysis.

Method of calculating biodegradation: Wax was assumed to have a mean composition of $[\text{CH}_2]$ for the purpose of calculating ThCO_2 (3.14 mg CO_2/mg wax). The report does not include the mechanics of calculation of the mineralization endpoint. Total hydrocarbon remaining at test termination was determined by area integration of the chromatograms, and primary biodegradability was determined by comparing the amount of hydrocarbons at the end of the test with the amount on wax-dosed filters prepared at the start of the test.

Other: Test substance was incubated in the inoculated mineral medium in sealed vessels containing a vial of 0.4 M NaOH (5 ml) suspended in the headspace above the medium (similar to EPA 835-3100). Carbon dioxide evolution resulting from mineralization of the test substance was trapped in the base for periodic quantitation. Base was renewed at each sampling period. GC analysis for parent compound was carried out on the solid phase of the test medium at study termination.

Reliability

: (2) valid with restrictions

(27)

Date 8/6/02

Type : Aerobic
Inoculum : Naturally-occurring leaf-litter and soil biota (microbes and invertebrates)
Contact time : 6 month
Year : 1989
Test substance : CAS 8002-74-2 and CAS 63231-60-7
Result : Decomposition in the 5 mm mesh bag, which were exposed to invertebrates as well as microbes, proceeded at a higher rate than in the 45 μ m bags. Decomposition in the 5 mm mesh bags was nearly complete within 13 weeks in the autumn/winter test and within 26 weeks in the spring/summer test, while in the 45 μ m bags 25 - 50% was still left after 6 months, based on visual observation. Wax residue analyses also indicated more rapid degradation in the cold-weather experiment.

Waxed and non-waxed (control) paper decomposed at the same rate.

Paraffin wax residue analysis showed after 6 months a complete or nearly complete degradation of the samples in the 5 mm mesh bags (the 52/54 paraffin wax showed 10% residues remaining after the spring/summer experiment and 0% after the autumn/winter experiment).

In the 45 μ m bags, wax residues remaining at the end of the summer exposure were 30 - 50% for the paraffins and intermediate wax, and 60% for the microcrystalline wax. After winter exposure, paraffin wax residues were 10 - 30% of initial, intermediate wax is reported as 80% of initial, and microcrystalline wax residues were 40% of initial. The winter value for the intermediate wax appears incorrect based on the chromatograms, which show smaller peaks for the winter vs. the summer analyses (same scale for both).

Test condition : Inoculum: Waxed paper was placed in nylon bags of different mesh size (45 μ m or 5 mm) to allow colonization by either microbes alone or by microbes and soil fauna. Leaf litter served as the source of the inoculum, and was placed in a layer over the mesh bags at the start of the test.

Concentration of test chemical: Approximately 20 mg of wax per mesh bag.

Temp of incubation: Ambient forest litter layer temperatures. Testing was carried out during two different seasons: spring/summer (April - October 1989) and autumn/winter (November 1989 - May 1990)

Dosing procedure: Each mesh bag contained four 2 x 2 cm squares of waxed paper, which were dried and weighed before they were placed in the bags. The squares were arranged in a single layer within the bags (10 x 10 cm) to avoid sticking together.

Sampling frequency: Samples were retrieved monthly and decomposition of the squares was estimated visually. The remaining sample material was then removed from the bags, cleaned, dried (50 °C) and weighed.

Controls: Non-waxed paper was used as a negative control.

Analytical method:

- 1) Physical decomposition of paper: Each piece of paper was assessed visually according to the scale 100%, 75%, 50%, 25%, 5%, and 0%

decomposition.

- 2) Wax residues were measured by extracting paper with warm heptane and the volume of extract adjusted prior to GC-FID analysis. To prevent interference of the analysis by the mesh bags, soil particles, and base paper, a cleanup step with aluminum oxide was used and as much of the bag material as possible was removed before extraction. The squares (or remnants thereof) from each treatment were pooled before extraction.

Method of calculating biodegradation: The extent of paper decomposition was determined by averaging the visual percent decomposition scores of the four squares. The degradation of the wax was calculated from the analysis of samples taken at the start of the test, combined with analyses of uncoated paper and of field blanks for determination of background interference. Weight differences were not used, as artifacts such as soil particles could not be removed from the waxed surfaces without removing the wax or destroying the paper.

Other: Two grades of paraffin wax, 52/50 and 58/60, intermediate wax, and microcrystalline wax were tested.

Conclusion

- : Waxed paper decomposes at about the same rate as unwaxed paper. Soil invertebrates contribute significantly to the decomposition of waxed paper in leaf litter. Decomposition of waxed paper occurs more rapidly during the autumn/winter, when there is a fresh layer of leaf litter on the ground, than during the spring/summer, when the last fall's leaf litter has been largely reduced to humus.

Reliability

- : (2) valid with restrictions, since positive control data not reported

(26)

Type : Aerobic
Inoculum : Unacclimated domestic sewage sludge supernatant and forest soil
Contact time : 137 day
Deg. Product : No
Method : Shake flask test
Year : 1989
GLP : No data
Test substance : Paraffin wax CAS 8002-74-2
Result : Degradation % after time: 55 % of ThCO₂ after 31 days;
 98.5% after 137 days

Kinetic (for sample, positive and negative controls):

Reference (cellulose) 88.7% after 31 days
 Test substance - 55% (31d); 98.5% (137 d)

Test condition : **Inoculum:** Soil was collected from a state park in central NJ, and sewage sludge was obtained from a domestic sewage treatment plant in Pennington, NJ. The sludge was aerated for 30 minutes and allowed to settle for an additional 30 minutes before the supernatant was withdrawn and filtered through #1 filter paper prior to use as the sewage inoculum. Filtrate was used at a rate of 25 ml/l of test medium (2.5%). Soil was added directly to each test flask at a rate of 0.1 g/l.

Concentration of test chemical: Test substance loading was approximately 10 mg carbon/l of medium.

Temp of incubation: 25 °C

Dosing procedure: Test material was added by direct addition of 11.8 mg grated wax to each test flask. Reference material (cellulose) was also weighed (25 mg) and added to the reference flasks to provide 10 mg C/l.

Sampling frequency: Carbon dioxide production was monitored after 2, 4, 7, 10, 17, and 24 days, and approximately weekly thereafter through day 137.

Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Cellulose was used as the positive control.

Analytical method: Carbon dioxide produced by mineralization of the test substances was absorbed in 0.2 N KOH solution in cuvettes in the headspace of the test vessels. CO₂ production was measured by titrating residual base with 0.2 N HCl.

Method of calculating biodegradation: Wax was assumed to contain 85% carbon for the purpose of calculating ThCO₂ wax). Average titration volumes at each sampling point were corrected for average blank volumes, then the amount of carbon dioxide produced was divided by ThCO₂ to determine percent biodegradation.

Conclusion : Not readily biodegradable; inherently biodegradable and extensively biodegradable in long-term exposures

Reliability : (2) valid with restrictions. Unable to determine GLP status. Study report is in the form of a memo from which some details are lacking. Same details (e.g., temperature log) are also lacking from the raw data provided with the

report

(5)

Type : Aerobic
Inoculum : Unacclimated domestic sewage sludge supernatant and forest soil
Contact time : 137 day
Result : Extensively biodegraded in long-term test
Deg. Product : No
Method : Shake flask test
Year : 1989
GLP : No data
Test substance : Microcrystalline wax CAS 63231-60-7
Result : Degradation % after time: 27 % of ThCO₂ after 31 days;
67.2% after 137 days

Kinetic (for sample, positive and negative controls):

Reference (cellulose) 88.7% after 31 days

Test substance - 27% (31d); 67.2% (137 d)

Test condition : Inoculum: Soil was collected from a state park in central NJ, and sewage sludge was obtained from a domestic sewage treatment plant in Pennington, NJ. The sludge was aerated for 30 minutes and allowed to settle for an additional 30 minutes before the supernatant was withdrawn and filtered through #1 filter paper prior to use as the sewage inoculum. Filtrate was used at a rate of 25 ml/l of test medium (2.5%). Soil was added directly to each test flask at a rate of 0.1 g/l.

Concentration of test chemical: Test substance loading was approximately 10 mg carbon/l of medium.

Temp of incubation: 25 °C

Dosing procedure: Test material was added by direct addition of 11.8 mg grated wax to each test flask. Reference material (cellulose) was also weighed (25 mg) and added to the reference flasks to provide 10 mg C/l.

Sampling frequency: Carbon dioxide production was monitored after 2, 4, 7, 10, 17, and 24 days, and approximately weekly thereafter through day 137. Controls: Yes (blank and positive controls per guideline); abiotic and toxicity checks were not included. Cellulose was used as the positive control.

Analytical method: Carbon dioxide produced by mineralization of the test substances was absorbed in 0.2N KOH solution in cuvettes in the headspace of the test vessels. CO₂ production was measured by titrating residual base with 0.2 N HCl.

Method of calculating biodegradation: Wax was assumed to contain 85% carbon for the purpose of calculating ThCO₂ wax). Average titration volumes at each sampling point were corrected for average blank volumes, and then the amount of carbon dioxide produced was divided by ThCO₂ to determine percent biodegradation.

Reliability : (2) Valid with restrictions. Unable to determine GLP status. Study report is in the form of a memo from which some details are lacking. Same details (e.g., temperature log) are also lacking from the raw data provided with the report

3. Environmental Fate and Pathways

Id Waxes

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(5)

Inoculum : Activated sludge, domestic
Contact time : 28 day
Method : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year : 1995
GLP : Yes
Test substance : Slack wax (petroleum), hydrotreated CAS 92062-09-4
Result : By day 28, 40% degradation of the test material was observed and indicated that the test material was inherently biodegradable. By day 5, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted. Biodegradation was based on net oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
SN 60	50.20, 34.54, 33.92	39.55
Na Benzoate	82.04; 72.88	77.46

Test condition : * replicate data
 Fresh activated sludge was obtained one day prior to test initiation, and homogenized in a blender for two minutes. After allowing the sample to settle for approximately 30 minutes, the homogenated supernatant was decanted, avoiding carry-over of solids. Microbial activity of an aliquot of the filtered supernatant was 1E6 CFU/ml, which was determined using microbial agar dip slides. Activated sludge supernatant was added to the test medium at 10 ml/l, and the inoculated medium was continuously aerated with CO₂-free air until the next day when the test systems were prepared. Test medium consisted of glass distilled water and mineral salts (phosphate buffer, ferric chloride, magnesium sulfate, calcium chloride). Test vessels were 1L glass flasks located in a water bath and electronically monitored for oxygen consumption. Test material was tested in triplicate; controls and blanks were tested in duplicate. Test material (Slack wax (petroleum), hydrotreated) concentration was approximately 37 mg/l, equivalent to a theoretical oxygen demand (ThOD) of 127 mg/l. Test material was weighed onto a Gelman type A/E 13 mm glass fiber filter, which was then added to each respirometer flask. Sodium benzoate (positive control) concentration was 53.54 mg/l, and was added using an aliquot of a stock solution.
 Test temperature was 22 ± 1° C. All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Reliability : (1) valid without restriction

(22)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

: See remarks in section 4.9 below

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

: See remarks in section 4.9 below

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

: See remarks in section 4.9 below

4.9 ADDITIONAL REMARKS

: The physical size and number of carbon atoms in petroleum waxes and related materials severely limits the ability of these materials to be taken up into living organisms. It is accepted that the ecotoxicity of alkanes of carbon number greater than C₁₀ are not acutely toxic to aquatic organisms at their limit of solubility in water (Adema, 1986). The petroleum waxes, containing hydrocarbons greater than C₁₃, would not be expected to cause acute toxicity to aquatic organisms. The results of toxicity tests with lubricant base oils, which have similar hydrocarbon ranges and some structures in common, show no acute toxicity to freshwater fish, invertebrates, or algae and no chronic effects to aquatic life at concentrations below 1 mg/l. (CONCAWE, 1997)

(4) (12) (13)

: The values of log Kow for individual hydrocarbons increase with increasing carbon number within homologous series of generic types. Quantitative structure activity relationships (QSAR), relating log Kow values of single hydrocarbons to toxicity, show that water solubility decreases more rapidly with increasing Kow than does the concentration causing effects (Abernathy, et al, 1988; Donkin, et al, 1991). This relationship varies somewhat with species, but it follows that there is a log Kow limit for hydrocarbons, above which, they will not exhibit acute toxicity; this limit is at a log Kow value of about 4 to 5 (Abernathy, et al, 1988; Donkin, et al, 1991). It has been confirmed experimentally that for fish and invertebrates, paraffinic hydrocarbons with a carbon number of 10 or higher (log Kow >5) show no acute toxicity (Adema, 1986) and that alkylbenzenes with a carbon number of 14 or greater (log Kow >5) similarly show no acute toxicity (Adema, 1991). From these well-demonstrated solubility 'cut-offs' for acute toxicity of hydrocarbon substances, which directly relate to their physico-chemical properties, it is clear that the same should hold for complex petroleum substances. QSAR equations for chronic toxicity also suggest that there should be a point where hydrocarbons with high log Kow

values become so insoluble in water that they will not cause chronic toxicity, that is, that there is also a solubility cut-off for chronic toxicity (McCarty, L.S. et al, 1991; European Union, 1996). Thus, paraffinic hydrocarbons with carbon numbers of greater than 14 ($\log K_{ow} > 7.3$) should show no measurable chronic toxicity. The existence of this cut-off for chronic toxicity is supported for petroleum substances by the numerous chronic toxicity studies reported on lubricant base oils, which demonstrate that for these substances which are composed primarily of alkanes and naphthenes of C15 and greater, no evidence of chronic toxicity is seen (CONCAWE, 1997). Further evidence to support this generalization is provided by a lack of chronic toxicity for hydrocarbon based solvents (CEFIC, 2000)

(1) (3) (4) (10) (12) (16) (19) (39)

- : In February of 2001 discharge of slack wax to national parks along British Columbia (Canada) coastline occurred during tank washing activities, impacting approximately 100 km of Pacific Rim National Park beach. Canadian Wildlife Service (a branch of Environment Canada) and the Department of Fisheries and Oceans biologists agreed that the risk of acute toxicity to aquatic life in the area was minimal based on the low solubility of the components in the wax and given that the BC Parks staff observed no significant environmental impacts. Generally the consensus was that the material was relatively inert and would likely pose little environmental damage.

(21)

5.1.1 ACUTE ORAL TOXICITY

Type : LD₅₀
Species : Rat
Strain : No data
Sex : Male/female
Number of animals : 10
Vehicle : Arachis oil
Value : > 5000 mg/kg bw
Year : 1976
GLP : No data
Test substance : R 9071 is a paraffin wax that was prepared as solutions in arachis oil for oral dosing. Two concentrations (20 and 100 mg/ml) were prepared for the two dose levels tested.
Method : Paraffin wax was administered orally as a solution in arachis oil to groups of 5 male and 5 female rats at dose levels of 1 and 5 g/Kg. The rats were observed for clinical signs of toxicity for the following 7 days. On the seventh day the animals were weighed, then killed and autopsied.
Result : There were no clinical signs of toxicity during the seven day observation period and growth rates were normal. There were no mortalities and no macroscopic changes were observed at autopsy. The LD₅₀ was found to be greater than 5g/Kg.
Reliability : (1) valid without restriction
 Although there is no indication that the study was carried out according to GLP, it nevertheless is a reliable study and full details are provided in the laboratory report.

(31)

Type : LD₅₀
Species : Rat
Strain : No data
Sex : Male/female
Number of animals : 10
Vehicle : Arachis oil
Value : > 5000 mg/kg bw
Year : 1976
GLP : No data
Test substance : R 9269 is a microcrystalline wax that was prepared as solutions in arachis oil for oral dosing. Two concentrations (20 and 100 mg/ml) were prepared for the two dose levels tested.
Method : Microcrystalline wax was administered orally as a solution in arachis oil to groups of 5 male and 5 female rats at dose levels of 1 and 5 g/Kg. The rats were observed for clinical signs of toxicity for the following 7 days. On the seventh day the animals were weighed, then killed and autopsied.
Result : There were no clinical signs of toxicity during the seven day observation period and growth rates were normal. There were no mortalities and no macroscopic changes were observed at autopsy. The LD₅₀ was found to be greater than 5g/Kg.
Reliability : (1) valid without restriction
 Although there is no indication that the study was carried out according to GLP, it nevertheless is a reliable study and full details are provided in the

laboratory report.

(32)

5.1.2 ACUTE INHALATION TOXICITY**5.1.3 ACUTE DERMAL TOXICITY**

Type	: LD ₅₀
Species	: Rabbit
Strain	: No data
Sex	: No data
Vehicle	: Petrolatum
Value	: > 4000 mg/kg bw
Year	: 1972
GLP	: No
Test substance	: Paraffin wax/Petrolatum (50/50)
Method	: Method is not described.
Remark	: The report does not provide sufficient information to fully evaluate the study.
Reliability	: (4) Not assignable This information is taken from a published safety review conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified.

(18)

5.2.1 SKIN IRRITATION

Species	: Rabbit
Concentration	: Undiluted
Exposure	: Occlusive
Exposure time	: 24 hour(s)
Number of animals	: 9
Result	: Not irritating
Year	: 1984
GLP	: No data
Remark	: An expert panel on cosmetics reviewed the skin irritation data and reported: <ul style="list-style-type: none"> • An undiluted paraffin wax was non-irritant in a 24 hour occluded patch test in rabbits • A microcrystalline wax was slightly irritating in a 24 hour occluded patch test
Result	: The report contains the following statement: A sample of 100% paraffin wax was applied full strength under a single closed patch to the skin of 9 rabbits. No irritation developed. Three samples of 50% paraffin in petrolatum were tested in repeated, open patch applications to 6 rabbits. Two samples produced erythema in four animals that lasted three days,

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Reliability

and one produced erythema in one rabbit that lasted two days.
No other details are provided.
: (4) not assignable
This information is taken from a published safety review conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified.

(18)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : 50 %
Dose : 0.1 ml
Exposure Time : 72 hour(s)
Comment : Not rinsed
Number of animals : 6
Result : Slightly irritating
Year : 1984
GLP : No data
Result : The publication states:

Four 50% solutions of paraffin in petrolatum were each instilled into the eyes of six albino rabbits with no rinse. Eyes were observed for irritation for three days. Two of the samples caused mild irritation in one rabbit on day 1; the other samples were not irritating.

Reliability

: (4) not assignable
This information is taken from a published safety review conducted by an expert panel. Few experimental details are provided and the quality of the studies and the panel's conclusions cannot be verified.

(18)

5.3 SENSITIZATION

No data

5.4 REPEATED DOSE TOXICITY

Species : Rat
Sex : Male/female
Strain : Fischer 344
Route of admin. : Oral feed
Exposure period : 90 days
Frequency of treatment : Continuous in food
Doses : 0.002, 0.02, 0.2 & 2.0% in the diet
Control group : Yes, concurrent no treatment
Method : OECD Guide-line 408 "Subchronic Oral Toxicity - Rodent: 90-day Study"
Year : 1992
GLP : Yes
Test substance : Three finished waxes and six mineral oils were tested in a series of three studies.

Only the information on waxes is included here.
The 3 finished waxes were:

Low melting point wax (LMPW)

High melting point wax (HMPW)

High sulphur wax (HSW)

The characteristics of these waxes has been published elsewhere (CONCAWE, 1993).

The waxes were powdered and incorporated in the diet at a concentration of 10% wt. This concentrate was further diluted with control diet to achieve test diets containing 2.0, 0.2, 0.02 and 0.002% wax. Analytical studies were carried out to ensure stability of wax in the diet and homogeneity of mixing. Throughout the study diets were analyzed for mineral hydrocarbon content.

An extra control diet containing 2.0% coconut oil was also prepared and this was analyzed throughout the study.

Results of analytical measurements throughout the study demonstrated that dietary mixing had been adequate and that dietary levels were within acceptable limits

Method : Three separate studies were carried out at the same time. In the main study, groups of 20 male and 20 female rats were fed diets containing one of 3 different waxes at dietary concentrations of 0.002, 0.02, 0.2 and 2.0% for 90 days. Further groups of 60 male and 60 females were fed untreated control diet. Additionally groups of 20 rats of each sex were fed diets containing 2.0% coconut oil.

In a reversibility study, an extra group of 10 rats of each sex was fed diets containing one of the 3 different waxes at the 2.0% level or coconut oil at 2%. Groups of 30 rats of each sex served as controls for this reversibility study.

To determine tissue levels of hydrocarbons a study was also

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included in which 5 rats of each sex were fed diets containing one of the 3 waxes or coconut oil at the 2.0% dietary level for 90 days. Extra groups of rats (5 of each sex) were fed control diet or coconut oil or one of the three waxes for 90 days followed by exposure to control diet only for a further 28 days.

In all three studies, animals were monitored for weight, food intakes and clinical condition throughout. An ophthalmic examination was performed prior to treatment and prior to necropsy on the animals in the main study and those for the study of reversibility.

A full necropsy was performed on the main and reversibility study animals and a full range of hematological parameters were measured on blood samples taken from the animals. Clinical chemical measurements were also made on serum separated from the blood samples. A selection of organs was weighed and a range of tissues retained for subsequent histopathological examination. All tissues from the high dose group and control groups were examined by light microscopy. Additionally the liver, lymph nodes, spleen, kidney, small intestine and lung were examined from all the intermediate dose groups.

Mineral hydrocarbon levels were measured in a limited number of tissues in those animals designated for tissue level determinations.

Remark

- : The purpose of this study was to assess the safety in use of a variety of oils and waxes for food contact applications.

As a follow up to this study additional studies were carried out on other finished wax samples and the results are summarized in the table below.

The severity and incidence of the responses were related to the average molecular weights of the materials tested; the lower molecular weight materials causing the most severe effects (CONCAWE 1993).

Sample	Viscosity @ 100°C (mg/kg/day) (cSt)	Carbon Chain Length	Average Mol. Weight	NOAEL
LMPW	3.3	19-42	375	<2
Blend	8	19-80	470	<2
IMPW	6.3	21-49	480	<2
HSW	13.7	20-74	600	2000
HMPW	15.4	22-80	630	2000

LMPW: Low melting point finished wax

Blend: Blend of LMPW and HMPW

IMPW: Intermediate melting point finished wax

HSW: High sulfur wax

HMPW: High melting point finished wax

The findings from the above studies allowed the EU Scientific Committee for Food (SCF 1995) to set ADIs for the high sulphur (HSW) and high molecular weight waxes (HMPW), but not for the lower molecular weight materials since for these NOELS had not been established.

A further study has also been carried out in which Low Melting Point Wax was fed to F-344 and Sprague Dawley rats at dietary concentrations of 0.2 and 2.0% in the diet for 90 days.

The findings in the F-344 rats were essentially similar to those found in the studies summarized above but the Sprague Dawley rat was found to be a less sensitive strain.

The only effects of treatment seen were an increase in mesenteric lymph node weight and microscopic findings in the same tissue (microgranulomas and reticuloendothelial cell hyperplasia). These effects were less severe and less frequent than those seen in the F-344 rats.

Result

: The results of a series of dietary studies done on multiple samples of food grade finished waxes are reported here.

Only minor treatment-related effects were observed in those animals fed either high sulfur wax or high molecular weight wax when compared to controls and then only at the highest dose level. Furthermore, for these two waxes, hydrocarbon levels in the tissues were no greater than those of the controls fed untreated diet.

Although growth rates, food intakes and clinical condition of animals fed LMPW were unaffected by exposure, there was a spectrum of changes that occurred as follows.

Organ weight changes were recorded in both sexes. Liver and spleen weights (absolute & relative) were increased at the 2 and 0.2% dose levels. Although some reduction was observed after the reversal period in the 2% dose groups, they were still higher than the corresponding controls.

Mesenteric lymph node weights were only available for the high dose level animals and these were increased following exposure to LMPW. Although the lymph node weights had reduced in the reversibility group they had not returned to normal by the end of the reversibility period.

The following hematological changes occurred at the dose levels shown for those animals fed LMPW:

Parameter	Dietary concentration	
	0.2%	2%
<u>Males</u>		
Hemoglobin content	Reduced (2%)	Reduced (2%)
MCH	Reduced (2%)	Reduced (2%)
Neutrophils	Increase (22%)	Increase (23%)
Platelets	Reduced (7%)	Reduced (13%)
<u>Females</u>		
Hemoglobin content	Reduced (6%)	

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Erythrocyte count	Reduced (4%)	
Hematocrit	Reduced (4%)	
Reticulocytes	Increase (43%)	
Leucocyte count	Increase (26%)	Increase (48%)
Neutrophils	Increase (45%)	Increase (89%)
Lymphocytes	Increase (18%)	Increase (29%)
Monocytes	Increase (35%)	Increase (103%)
Eosinophils	Increase (41%)	
Basophils Actual value	0.003	0.004
(Control value = 0)		
Platelets	Reduced (14%)	Reduced (16%)

There were raised serum liver enzyme levels in the highest two dose groups of females but only at the highest dose in males. The enzymes affected were ALA, ALAT, ASAT and Gamma-GT. Serum bilirubin was also elevated in the highest dose group of females. Albumin/globulin ratios were reduced in the females at the highest 2 dose levels and in the highest dose level only for the males.

No mineral hydrocarbons were found in the kidneys of rats fed LMPW. However, it was found in the perirenal fat, liver and lymph nodes. After the reversal period mineral hydrocarbon was still found in these tissues, albeit at lower concentrations.

Histopathological lesions were observed in many tissues and were of a severity and nature consistent with the age of the animals and were not considered treatment-related. However, lesions in the liver, mesenteric lymph node, Ileum & jejunum and heart were considered compound-related. These were as follows:

Liver

Granulomas were observed in the livers of male and female rats at the highest 2 dose levels. At the highest dose centrilobular vacuolation was also observed. After the one month reversal period, granulomas were still present at the same incidence but their severity was less.

Mesenteric lymph node

The lymph node lesions comprised focal collections of slightly vacuolated macrophages in the cortical region and after one month's reversal these were reduced in severity. Such lesions occurred to varying degrees of severity at all dose levels.

Ileum & jejunum

There was an increased incidence in macrophage accumulation in Peyer's Patches in both sexes at the highest two dose levels. There was also an increase in macrophage infiltration of the lamina propria in the high dose females.

Heart

A focal inflammatory lesion was observed within the cusps of the mitral valve. The lesion was characterized by an increased cellularity of the valve

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Reliability

with destruction of the fibrous core. The lesion was observed in 11/20 males and 11/20 females at the highest dose level and 5/20 females at the 0.2% group. Following the 28-day reversal period there was still an increased incidence of the lesion but this was less than that at the end of the 90-day feeding study.

: (1) valid without restriction
Study conducted to GLP and thoroughly reported.

(7) (8) (9)

5.5 GENETIC TOXICITY 'IN VITRO'

: No data available

5.6 GENETIC TOXICITY 'IN VIVO'

: No data available

5.7 CARCINOGENITY

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 80 weeks
Frequency of treatment : Twice weekly
Doses : 50 mg/application
Result : Negative
Control group : Untreated control and positive control (BaP)
GLP : No
Test substance : Slack wax CAS No. 64742-61-6
The sample was tested twice in the study summarized by Kane et al.

Method : 50 mg melted slack wax was painted on the skin of 50 individually housed male mice, twice weekly for 80 weeks. The animals were shaved bi-weekly with electric clippers and the test material applied to the shaven intrascapular region. Treatment was continued for 80 weeks. A concurrent negative untreated control and a positive control (benzo-a-pyrene) was included in the study. The study was repeated using 25 mg/application, twice weekly.

Remark : This report is a summary of results from an extensive program of studies. Consequently, not all the experimental details have been presented. The authors state that such details are available in the original laboratory reports.

Result : No skin tumors developed in any of the mice to which slack wax had been applied in either of the studies. The responses in the control groups are not reported.

5. Toxicity

Id Waxes

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Reliability

: (4) Not assignable
The report summarizes data from many studies and does not contain sufficient detail for a full evaluation.

(34)

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : Dermal
Exposure period : 80 weeks
Frequency of treatment : Twice weekly
Doses : 50 mg/application
Result : Negative
Control group : Untreated control and positive control (BaP)
GLP : No
Test substance : Petrolatum CAS No. 8009-03-8
Method : 50 mg petrolatum was painted on the skin of 50 individually housed male mice, twice weekly for 80 weeks.
 The animals were shaved bi-weekly with electric clippers and the test material applied to the shaven intrascapular region.
 Treatment was continued for 80 weeks.
 A concurrent negative untreated control and a positive control (benzo-a-pyrene) was included in the study.
 The study was repeated using 25 mg/application, twice weekly.

Remark : This report is a summary of results from an extensive program of studies. Consequently, not all the experimental details have been presented. The authors state that such details are available in the original laboratory reports.

Result : No skin tumors developed in any of the mice to which petrolatum had been applied in either of the studies. The responses in the control groups are not reported.

Reliability : (4) not assignable
 The report summarizes data from many studies and does not contain sufficient detail for a full evaluation.

(34)

Species : Mouse
Sex : Male/female
Strain : Swiss
Route of admin. : Dermal
Exposure period : Lifetime
Frequency of treatment : Twice weekly
Doses : Approximately 60 μ l per application
Result : Negative
Control group : Yes, concurrent vehicle
Year : 1966
GLP : No data
Test substance : 15% solution of Amber Petrolatum (NF Grade) in isooctane
Method : Three drops (approximately 60 μ l) of a 15% solution of amber petrolatum in isooctane was applied to the shaven skin of the mice, twice weekly for their lifetimes.
 30 male and 40 female mice were treated in this way.
 A group of 50 males and 50 females served as vehicle controls and received 60 μ l of isooctane twice weekly for the lifespan of each animal. Animals were housed

Result

in groups of not more than 10 per cage.

The occurrence of skin tumors and other lesions in the treated area and other visible lesions was noted and their progression recorded.

Histological confirmation of each lesion was confirmed after autopsy of the respective animals.

: Treatment with petrolatum caused moderate epidermal hyperplasia.

The authors state that the incidence of internal tumors appeared within the limits observed in the control animals.

Treatment did not appear to affect survival when compared to controls as follows:

Group	Survival (%) at weeks		
	30	50	70
<u>Petrolatum</u>			
Females	90	77	53
Males	93	83	35
<u>Controls</u>			
Females	90	80	64
Males	90	54	32

The skin tumor incidence is summarized below for the control and petrolatum groups. No data are included here for the various extracts of petrolatum that were tested, even though such data were given in the publication reviewed.

Animals	Total number of			Latency (weeks)
	Tumors	Carcinomas	Regressions	
<u>Petrolatum</u>				
Females				
1	2*	-	1	100
Males				
2	3**	-	2	69
<u>Solvent</u>				
Females				
-	-	-	-	-
Males				
2	2	1	-	63

* one papilloma on eyelid

** one papilloma under chin

Reliability

: (2) valid with restrictions

The study was designed only to investigate skin carcinogenicity and consequently detailed pathological findings are not available. Detailed findings (histopathological) are not included in the paper, but the authors make reference to a source of such data.

(36)

Species	: Mouse
Sex	: Male/female
Strain	: Swiss
Route of admin.	: Dermal
Exposure period	: Lifetime
Frequency of treatment	: 3 times weekly
Doses	: 3 drops
Result	: Negative
Control group	: Yes, concurrent no treatment
Year	: 1962
GLP	: No data
Test substance	: 5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents Each of the 5 waxes was dissolved in warm benzene to achieve 15% solutions. These were warmed in a water bath prior to application to the skin. Additionally a benzene solvent control was included in the study as well as an aromatic extract (in is-octane) of one of the waxes and a 15% solution in benzene of a chromatographed wax.
Method	: 3 drops (approximately equivalent to 0.05 ml) of the solution of wax or the solvent control was applied to the skin of the intrascapular region over an area of approx. 2 X 2 cm. This treatment was continued 3 times weekly to groups of mice throughout the experiment. Observation was continued until spontaneous death or until the animals were killed when dying. All mice were subjected to a complete autopsy followed by a histological examination of all abnormal tissue. Group sizes were approximately 60 male and 30 female for each wax sample and 140 mice of each sex for controls.
Result	: Survival rates of the mice were similar for treated and control animals with a better survival among females than males. Some desquamation and epilation occurred in the treated areas of skin after the first few applications and this persisted throughout the study. Histologically, moderate epidermal hyperplasia was observed in both treated and control animals. The wax treated animals also had some focal areas of hyperplasia of the sebaceous glands. No degenerative or necrotic changes were observed.

The skin tumor incidences are shown in the following table. (overleaf)

5. Toxicity

Id Waxes

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No. of mice	Benign papillomas	Malignant carcinomas	Sebaceous gland adenomas	Other
<u>Wax 2</u>				
61 M	1			
30 F				
<u>Wax 8</u>				
61 M	3	1		
31 F	1			
<u>Wax 12</u>				
58 M	4		1	1
34 F	1		1	
<u>Wax 15</u>				
57 M	2			
30 F	1			
<u>Wax 20</u>				
61 M	1		2	
36 F	1		2	
<u>Benzene</u>				
59 M		1		
35 F	1			

A number of other tumors were also observed at autopsy (mainly lung adenomas, mammary carcinomas and malignant lymphomas) but these were found in all groups and their incidence was similar in wax treated groups and controls. The authors judged that these studies were negative.

Reliability

- : (2) valid with restrictions
Although not conducted to GLP, the study was nevertheless, robust and is acceptable for the purpose of assessing the skin carcinogenicity potential of paraffin wax solutions in benzene.

(45)

Species : Mouse
Sex : Male
Strain : White albino
Route of admin. : Dermal
Frequency of treatment : Three times weekly for lifetime
Year : 1951
GLP : No
Test substance : Eight slack waxes and eight aromatic hydrocarbon extracts derived from the slack waxes were tested.
 [Because of the lack of detail in the publication it is not possible to establish which aromatic extract was derived from which specific slack wax].

The extracts were obtained by eluting, with an unspecified solvent, silica gel columns charged with the individual slack waxes. No additional information was provided on the preparation of the aromatic test materials. [However, in parallel studies on aromatic extracts collected from catalytically cracked oils, the investigators reported that the silica gel columns were eluted first with n-heptane to collect non-aromatic components of the oils and then with acetone to recover the aromatic components. In the parallel studies the recovered aromatics were tested on mice after evaporation of the acetone.]

Method : Approximately 15 mg of warmed test material were applied as a thin film by means of a small brush on Monday, Wednesday and Friday to the shorn scapular region of groups of 30 albino male mice. Test material application was continued until death. After tumors had appeared the test materials were applied around the viable base of the growths, not on their often "dead tops".

For each material at autopsy, sections were taken of representative tumors and any internal lesions of interest. These tissue sections were then examined microscopically.

For each test material a cancer and a tumor index was calculated as follows:

$$\text{Tumor index} = \frac{100 \times \text{Total No of animals in which tumors developed}}{\text{Original No. animals less No dead at 90 days without tumors}}$$

$$\text{Cancer Index} = \frac{100 \times \text{Total No animals in which cancer developed}}{\text{Original No less No. dead at 90 days from causes other than cancer}}$$

$$\text{Potency was calculated:} = \frac{\text{Cancer index}}{\text{Tumor index}}$$

Result : Results are summarized in the following two tables:

Slack waxes

Wax Sample	Oil (%) ¹	CI/TI at Days	
		250	450
145	25	4/23	8/10 ³
147	17	0/3	7/7
150	20	0/0	4/4
141	10	0/3	0/7
142	21	0/4	0/4
144	21	0/4	0/4
140	20	4/7	4/4 ³
146	12	0/0	4/4

Aromatic extracts

Sample	Aromatic (%) ²	CI/TI at Days	
		250	450
231	18	14/38	24/38
233	0	19/30	23/35 ⁴
235	12	17/35	17/43 ⁴
228	7	3/17	14/34
229	0	0/0	0/13
230	12	0/42	8/30 ^{3 5}
231	11	4/22	4/30 ⁴
232	8	0/8	4/10

¹ Oil content of the slack waxes (w/w)

² Aromatics content of the slack wax (w/w)

³ The lower tumor index (TI) at the later date is due to spontaneous disappearance of some papillomas

⁴ The experiment was discontinued after 335 days

⁵ The experiment was discontinued after 490 days

The authors concluded that the slack waxes showed only a low order of carcinogenicity at 250 days. However by 450 days every sample of slack wax had elicited papillomas and for 5 of them cancers as well.

The aromatic extracts on the other hand exhibited a greater potency. At 250 days all but one sample had produced papillomas and 5 samples had produced cancers. At 450 days all but one sample had elicited cancers and all had elicited papillomas.

The authors concluded that the carcinogenicity of slack wax

1. Can be attributed to the aromatic compounds found in the oils from which the waxes were pressed and which are retained on the waxes as impurities.
2. Is not due to paraffins.

Another study from the same laboratory (Dietz et al, 1952) on 11 slack waxes (it is unclear whether some were the same samples as in Smith et al, 1951) produced similar results. The tumor potency of each sample was low to marginal.

Reliability

: (4) not assignable

The study summarized here was conducted to identify the carcinogenic component(s) of slack waxes.

Although not conducted to GLP and lacking experimental details the study is important since it identifies the residual oil in the slack wax and not the paraffins as being responsible for carcinogenic activity.

(15) (46)

Species	: Rabbit
Sex	: Male/female
Strain	: New Zealand white
Route of admin.	: Dermal
Frequency of treatment	: Three times weekly
Control group	: Yes, concurrent vehicle
Year	: 1962
GLP	: No
Test substance	: 5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents Each of the 5 waxes was dissolved in warm benzene to achieve 15% solutions. These were warmed in a water bath prior to application to the skin. Additionally a benzene solvent control was included in the study.
Method	: Solutions of the waxes as well as the benzene alone were applied three times weekly to the shorn skin of the intrascapular region (approximately 10 X 10 cm) of 4 male and 4 female rabbits. Each application consisted of approximately 0.08 ml. The authors state that a few rabbits were added in some groups to compensate for death of other rabbits before one year of treatment. Specific details are not provided.
Remark	: This study had not been completed at the time of publication of a paper on the toxicity of petroleum waxes (Shubik et al). However, the information is useful in assessing the skin carcinogenicity of petroleum waxes since it provides data from an additional species.
Result	: Some reddening, desquamation and epilation of the painted skin area occurred after a few paintings with the wax solutions and the benzene alone; these changes persisted throughout the study without any notable modifications. 2 small skin papillomas were observed in the male group painted with one of the waxes. One of these papillomas developed after 48 weeks of treatment and was still present at the 105th week. The other papilloma developed after 93 weeks and regressed at the 110th week. No other skin lesions were found in any of the groups.
Reliability	: (4) Not assignable This study was not reported thoroughly, nor was it complete at the time of publication. However, it does provide supportive information from a species other than the mouse.

(45)

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Species : Rat
Sex : Male/female
Strain : FDRL
Route of admin. : Oral feed
Exposure period : 2 years
Frequency of treatment : Ad libitum
Doses : 5% in the diet
Result : Negative
Control group : Yes, concurrent no treatment
Year : 1965
GLP : No data
Test substance : Three blends of petrolatum were examined. They were as follows:

Blend A, a snow-white grade meeting USP XVI specifications. This sample was a blend in equal proportions of six commercially available materials, each meeting the US specification.

Blend B, a white petrolatum, somewhat darker than Blend A, but nevertheless meeting the USP XVI specification. This blend was also prepared as a mixture of six commercially available materials in equal proportions.

Blend C, a yellow petrolatum meeting NF XI specification. This blend was prepared as a mixture in equal proportions of 5 commercially available products.

The three blends were kept with minimum air space refrigerated in metal containers for the duration of the study.

Analytical characteristics of the blends were as follows:

Blend	UV absorptivity (290 mμ)	Lovibond color (2 in. cell)	Specific gravity (60 °C)	Melting point (°C)
A	0.136	2Y	0.830	53.5
B	0.424	12Y 0.5R	0.835	52.2
C	1.48	35Y 10R	0.844	51.3

Method : 50 rats of each sex, individually housed were fed diets containing 5% of one of three blends of petrolatum ad-libitum for two years. A group of 100 rats of each sex served as controls and were fed normal diet ad-libitum that had been supplemented with 1% vitamin mix and 0.2% Aurofac 10.

The animals were observed daily for appearance, behavior and survival.

Weekly measurements were made of body weight for the first

12 weeks of the study and biweekly thereafter. Weekly measurements were also made of food intake for the first 12 weeks for 10 rats of each sex fed the diets containing petrolatum and for 20 rats of each sex fed control diet.

At 12, 26, 52, 72 & 100 weeks the following determinations were made on representative animals from each of the groups: red cell count and/or hematocrit, total and differential white cell counts, hemoglobin content, blood glucose and blood urea nitrogen levels.

Rats that died and survivors at the end of the study were autopsied and the following organ weights were recorded: liver, kidneys, spleen, heart, adrenals, thyroids and pituitary.

For all rats that died, that were killed in a moribund state or from representative surviving animals at the end of the 2 year feeding period (10 of each sex in the petrolatum groups, 20 of each sex controls) the following organs were fixed and examined histologically: liver, spleen, stomach, large and small intestine, pancreas, kidney, urinary bladder, adrenal, thyroid gland, testis or ovary, salivary gland, lymph node, heart, lung, muscle, skin, spinal cord, brain, thymus, bone marrow and "growths of any description".

Result

: Growth rates were unaffected by exposure to petrolatum when compared to controls.

Although there were small statistically significant differences in food utilization values between control and some petrolatum exposed animals these were not of biological significance.

Survival at two years was unaffected when compared to controls. Survival of males was approximately 68% and that for females was 58%.

Neither hematological nor clinical chemical measurements were affected by exposure to any of the petrolatum samples either during or at the end of the study.

No differences were found at autopsy between petrolatum exposed and control animals. Furthermore, there were no histological changes that could be attributed to dietary exposure to petrolatum. Histological changes that occurred did so in both sexes and in all treatment and control groups and were considered to be ageing related.

Neither of the 3 petrolatum blends caused an increased tumor incidence in any tissue/organ examined.

**Test substance
Reliability**

:
: (2) valid with restrictions

This study is well conducted and reported, but was carried out prior to the need for GLP. Nevertheless the study is valid.

(42)

Date 8/6/02

Species	: Rat
Sex	: Male/female
Strain	: Sprague-Dawley
Route of admin.	: Oral feed
Exposure period	: 2 years
Frequency of treatment	: Continuous
Post. obs. period	:
Doses	: 5000mg/kg bw/day
Result	: Negative
Control group	: Yes, concurrent no treatment
Year	: 1962
GLP	: No
Test substance	: 5 waxes were selected from 36 samples on the basis of their ultraviolet absorptivity, representing the range of aromatic contents Each of the 5 waxes was ground into a powder and added to powdered diet and mixed in the proportion 1:9 w/w
Method	: Each of the five waxes was fed ad-libitum to male and female rats at a dietary concentration of 10% for 2 years. Additional groups of 140 male and 157 females were fed control diet. The rats inspected and weighed every second week and all gross lesions were recorded. This was continued until the rats died or were killed when dying and were then submitted to complete autopsy followed by histological examination of all abnormal tissue.
Result	: Survival rates and growth rates were unaffected by oral exposure to any of the waxes tested. A number of tumors were found in all groups at autopsy. The incidence of each tumor type was reported. The number of tumor bearing animals was similar to that of controls and furthermore the incidence of the various tumor types was also similar in treated and control animals. No other toxic effects were found at histological examination. The authors concluded that the five waxes were devoid of carcinogenic or other toxic action when fed at a level of 10% in the diet.
Reliability	: (2) Valid with restrictions Study not carried out according to GLP and only "abnormal" tissue examined histologically. Study provided supportive information only and could not be used as a definitive study.

(45)

Date 8/6/02

Species : Rat
Strain : BD I, BD III and W
Route of admin. : Various
Exposure period : Up to approximately 2.5 years
Frequency of treatment : Various
Year : 1953
GLP : No
Test substance : Various, including yellow vaseline
Remark : The following is taken from the method section of an English translation of the German report:

"
Liquid paraffin (DAB. 6) was injected into 30 rats, 2.5 ml once subcutaneously and intraperitoneally in a total dose of 9 ml per animal divided over 15 individual injections over a period of 40 weeks. Another 30 rats obtained the liquid paraffin in the food. The total dose was 136 ml/animal in 500 days.

Yellow vaseline (DAB. 6) was also injected after warming. Eight rats obtained 3 ml intraperitoneally and 26 rats 1 ml subcutaneously besides. All animals were observed until spontaneous death....."

The following is taken from the results section of the publication.

"
In the experiment with vaseline a tumor developed at the injection point after a latent period of 658 days. Histologically this tumor turned out to be an osteo-sarcoma....."

Reliability

(3) invalid

This study is of historical interest only and is included for completeness only.

(44)

Date 8/6/02

Species : Mouse
Sex : Male/female
Strain : Swiss Webster
Route of admin. : s.c.
Frequency of treatment : Single subcutaneous dose
Post. obs. period : 18 months
Doses : 100 mg
Result : Negative
Control group : Yes
Year : 1965
GLP : No
Test substance : Three blends of petrolatum were examined. They were as follows:

Blend A, a snow-white grade meeting USP XVI specifications. This sample was a blend in equal proportions of six commercially available materials, each meeting the US specification.

Blend B, a white petrolatum, somewhat darker than Blend A, but nevertheless meeting the USP XVI specification. This blend was also prepared as a mixture of six commercially available materials in equal proportions.

Blend C, a yellow petrolatum meeting NF XI specification. This blend was prepared as a mixture in equal proportions of 5 commercially available products.

The three blends were kept with minimum air space refrigerated in metal containers for the duration of the study.

Analytical characteristics of the blends were as follows:

Blend	UV absorptivity (290 mμ)	Lovibond color (2 in. cell	Specific gravity 60 °C)	Melting point (°C)
A	0.136	2Y	0.830	53.5
B	0.424	12Y 0.5R	0.835	52.2
C	1.48	35Y 10R	0.844	51.3

Method

Stripped lard was used as negative control substance.
: A single dose of 100 mg of one of the three petrolatum blends or stripped lard was administered subcutaneously into the intrascapular region of 28-day-old mice. 50 male and 50 female mice were used for each group and these were housed individually for the following 18-month observation period. The mice were allowed food and water ad-libitum. Growth, physical appearance and behavior were observed throughout the study and special attention was paid to the injection site.

Date 8/6/02

Result

Representative mice sacrificed at 9 months and all mice that died or were sacrificed at the end of the 18-month observation period were examined at autopsy for evidence of pathological change. Weights of liver, spleen and kidneys were recorded. After fixation, histological examination was made of: liver, spleen, stomach, small and large intestine, pancreas, kidney, urinary bladder, adrenal, thyroid, testis or ovary, salivary gland, lymph node, heart, muscle, lung, skin, spinal cord, brain, thymus and bone marrow and any macroscopically observed growths.

- : Growth rates, food intakes and food utilization was unaffected by s.c. administration of any of the petrolatum samples when compared to the control group. The males consumed slightly more food than the females, but there were no differences between the various treatment groups. Mortality was similar in the control and petrolatum groups and overall survival ranged between 12 and 24% at the end of the study (78 weeks). Liver, kidney and spleen weights were not affected by exposure to any of the petrolatum blends. Gross observations at autopsy were spread equally amongst all groups and were not specifically related to exposure to petrolatum. At about 7-9 months, there had been a significant rise in mortality in all groups and histopathological examination confirmed widespread leukemic infiltration with secondary septicemic involvement in some animals in all groups. Gross findings at the end of the study were consistent with ageing animals. The responses were largely either of a chronic inflammatory or fibrotic nature. Many of the observations in the lymphatic system showed chronic changes associated with the clearance of the foreign material that had been injected subcutaneously. There was no specific relationship between tumor incidence and the test material injected.

Reliability

- In conclusion, no toxic or carcinogenic response resulted as a consequence of the s.c. injection of a 100 mg dose of either of the 3 petrolatum blends.
- : (2) valid with restrictions
This study is well conducted and reported, but was carried out prior to the need for GLP. Although survival of mice was poor, nevertheless the study is considered valid.

(42)

Date 8/6/02

Species	:	Mouse
Sex	:	Male/female
Strain	:	Swiss
Route of admin.	:	s.c.
Exposure period	:	Lifetime
Frequency of treatment	:	Once only administration of test material
Post. obs. period	:	Lifetime
Year	:	1962
GLP	:	No
Test substance	:	Paraffin wax
Method	:	A single wax disc (2 cm. diameter, 2 mm. thick and weighing 0.5 g) was implanted subcutaneously in groups of approximately 45 male and 50 female Swiss mice. This was done for 5 different waxes. Additionally, 0.5 g of one of the waxes was implanted as a powder in a further group of 48 and 46 female Swiss mice. The animals and their controls were observed for their lifetimes.
Result	:	Tumors developed at the implantation sites of the wax discs. No tumors developed at the site s of the powdered wax.
Reliability	:	This finding is consistent with other reports on the tumorigenicity of implanted inert materials. It is generally believed that tumorigenicity at subcutaneous implantation sites is a function of the physical form of the material rather than of the material itself. If however, the material had been tumorigenic it would be expected that tumors would have developed at the site of the implanted powder. (2) Valid with restrictions Although the study was not GLP compliant it nevertheless was properly conducted and reported.

(45)

5.8 TOXICITY TO REPRODUCTION

No data

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

No data

5.11 EXPERIENCE WITH HUMAN EXPOSURE

**Memo
Remark****: Slack wax**

- : There are no published reports of acute effects in humans with slack waxes, but they are expected to be essentially non-toxic because both the residual oil and the wax components themselves are not acutely toxic.

There have been several reports of human occupational cancer amongst wax pressmen, during the preparation of paraffin wax (Hendricks et al, 1959; Lione and Denholm, 1959). In the process of wax pressing the unrefined or poorly refined oil was chilled and the solidified crude wax (slack wax) removed from the viscous oil on filter presses. This crude wax may have contained as much as 20-40% unrefined/poorly refined oil, which was reduced to less than 0.5% in subsequent processing. It should be noted that wax pressing is no longer used as a process and has been replaced by more modern techniques.

(29) (37)

**Memo
Remark****: Paraffin wax**

- : A review of the clinical studies with two undiluted paraffin waxes and formulated products containing various concentrations of paraffinic (5-16%) and microcrystalline (4.35-15%) waxes was published (Elder, 1984). These studies include a range of acute and repeat application tests in groups of humans for skin irritation and skin sensitization. All products gave, at most, slight erythema and none caused skin sensitization.

The widespread use in cosmetic and in cosmetic surgery over many years demonstrates the low toxicity of refined waxes and many guidelines exist for their safe use (Hjorth, 1987). Notwithstanding this, there are occasional reports of adverse effects with these products. Subcutaneous deposits, often referred to as paraffinoma, have been described frequently following injection of these materials under the skin but these are not normally associated with other progressive changes.

There has been one report where an outbreak of skin rashes was attributed to occupational exposure to wax fume (Halton & Piersol, 1994).

(18) (25) (30)

**Memo
Remark****: Petrolatum**

- : Despite the widespread use of petrolatum for many years as a vehicle in human skin patch testing, isolated cases of allergy to petrolatum have been reported. Nevertheless, petrolatum is still considered to be a good vehicle for patch testing. Fisher has concluded that although allergic reactions to petrolatum are rare, white, and not yellow petrolatum should be used as a vehicle in human skin patch testing.

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5. Toxicity

Id Waxes

Date 8/6/02

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